

Along the ecocline:

Physiological responses of Baltic Sea and coastal peatland benthic diatoms to abiotic variables

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“The highest function of ecology is the understanding of consequences”

– Frank Herbert

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Abbreviations

ANOVA	analysis of variance
ATP	Adenosine triphosphate
cf.	confer (likely to belong to the identified species)
CH ₄	Methane
Cl ⁻	Chloride ion
cm	centimeter
CO ₂	carbon dioxide
d	day
DOM	dissolved organic matter
DMSP	dimethylsulfoniopropionate
(e)DNA	(environmental) deoxyribonucleic acid
e.g.	exempli gratia (for example)
EPS	exopolymeric substances
et al.	et alia (and others)
f/2	a type of culture medium
FFA	free fatty acids
Fig.	Figure
(m)g	(milli)gram
gt	gigatons
h	hour
ha	hectare
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
ITS	internal transcribed spacer
i.e.	id est (that is)
LM	light microscopy
in prep.	in preparation
K ⁺	Potassium ion
km/km ²	kilometer/square kilometer
LED	light-emitting diode
(m)L	(milli)liter
(m)m	(milli)meter
mM	millimolar
MoBi	molecular biology
MP6	designated sampling station
MP7	designated sampling station
MX	Medium X
N	nitrogen
n	number of replicates per treatment
Na ⁺	Sodium ion
NaCl	Sodium Chloride

Abbreviations

NaH ₂ PO ₄ *H ₂ O	Sodium Phosphate
NH ₄ ⁺	Ammonium
NaNO ₃	Sodium Nitrate
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate
NPQ	non-photochemical quenching
NaSiO ₃ *H ₂ O	sodium silicate
NCBI	National Center for Biotechnology Information
NGS	next generation sequencing
P	phosphorus
p	p-value (significance)
PAR	photosynthetically active radiation
PFD	photon flux density
pH	negative decimal logarithm of the hydrogen ion activity in a solution
PI	photosynthesis-irradiance
PO ₄ ³⁻	Phosphate
PSII	photosystem II
(q)PCR	(quantitative) polymerase chain reaction
<i>rbcL</i>	Ribulose biphosphate carboxylase large chain precursor
RNA	ribonucleic acid
ROS	reactive oxygen species
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
s	seconds
S17	designated sampling station
S _A	absolute salinity
SAC ₂₅₄	spectral absorption coefficient at 254 nm (nanometer)
SD	standard deviation
SEM	scanning electron microscopy
SGD	submarine groundwater discharge
Si	Silica
SiO ₂	Silicon Dioxide
sp.	species
st.	strain
t CO ₂ eq ha ⁻¹ y ⁻¹	ton carbon dioxide effectiveness-equivalent per hectare and year
TAG	triacylglycerol
°C	degrees Celsius
μL	microliter
μmol	micromole
μM	micromolar
μS	microSiemens

Abstract

The southern Baltic Sea coast is strongly shaped by abiotic variables and is constantly changing. Due to the climate change-related sea-level rise and increased storm-surge events, terrestrial-marine exchange processes are increasing significantly. These processes are enhanced by renaturation of coastal peatlands through deconstruction of dunes and dikes, thereby facilitating exchange processes between the shallow Baltic Sea and the coastal peatlands. However, the ecophysiological consequences to the organisms in these contrasting environments are barely known. This applies especially to the microphytobenthos, a phototropic biofilm often dominated by diatoms, which, due to their function as primary producers, massively contribute to the biogeochemical cycles in both ecosystems.

This dissertation focused on the ecophysiological responses of unialgal diatom isolates from the microphytobenthos in the shallow southern Baltic Sea and adjacent coastal peatlands to the fundamental variables salinity, temperature, and light availability in their respective habitats, as well as the influence of potential mixing of waterbodies of the two ecosystems on growth rates and photosynthetic performance of the benthic diatoms.

Publications I and II described the wide photo-physiological plasticity of diatom strains from the Baltic Sea and coastal peatlands to the prevailing light conditions of their habitats. All diatom strains also showed euryhaline and eurythermal tolerances that greatly exceeded the prevailing temperature and salinity ranges in the two habitats.

In **publication III**, species-specific growth rates were determined in media with different proportions of Baltic Sea and peatland water. Peatland water significantly stimulated the growth of the Baltic Sea and peatland diatom strains. The Baltic Sea strains also showed dependency to higher salinities requiring at least brackish water conditions.

Finally, the results in **publications I and III** revealed a stimulating effect of peatland water on the specific growth rates of the benthic diatoms, indicating mixo- or heterotrophic capabilities related to the high availability of organic carbon in the peatland waterbodies.

In summary, this dissertation showed that due to their high photo-physiological plasticity as well as wide euryhaline and eurythermal tolerances, Baltic Sea and peatland diatoms are well acclimated to the fluctuating abiotic variables of their respective habitats. The data may indicate that benthic diatoms will thrive under the environmental changes resulting from renaturation of coastal peatlands or climate change.

Zusammenfassung

Die südliche Ostseeküste ist stark von abiotischen Variablen geprägt und verändert sich daher zusehends. Durch den klimawandelbedingten Meeresspiegelanstieg und verstärkten Sturmfluten nehmen vor Allem terrestrisch-marine Austauschprozesse stark zu. Begünstigt werden diese Austauschprozesse zusätzlich durch die Renaturierung von Küstenmooren, welche durch Rückbaumaßnahmen von Dünen und Deichen wieder verstärkt zwischen den Küstengebieten der Ostsee und den Küstenmooren ermöglicht werden. Die ökophysiologischen Folgen dieser Austauschprozesse sind für die dort lebende Organismen jedoch kaum bekannt. Dies gilt besonders für das Mikrophytobenthos, ein phototroper Biofilm, welcher oft von Diatomeen dominiert wird und durch seine Funktion als Primärproduzent massiv zu den biogeochemischen Kreisläufen beiden Ökosysteme beiträgt.

Diese Dissertation befasst sich daher damit, die ökophysiologischen Antworten von dominanten Diatomeenarten des Mikrophytobenthos aus dem Flachwasserbereich der südlichen Ostsee und anliegenden Küstenmooren auf grundlegende Variablen wie Salinität, Temperatur und Lichtverfügbarkeit zu untersuchen, sowie den Einfluss potenzieller Vermischungsprozesse der Wasserkörper beider Ökosysteme auf das Wachstum und die Photosyntheseleistung benthischer Diatomeen zu ermitteln.

In **Publikation I und II** zeigte sich eine breite photo-physiologische Plastizität der Ostsee- und Moorisolate gegenüber den vorherrschenden Lichtbedingungen ihrer Herkunftshabitate. Des Weiteren wiesen alle Diatomeenisolate euryhaline und eurytherme Toleranzen auf, welche die prävalierenden Temperatur- und Salzgehalte beider Habitate stark überschreiten.

In **Publikation III** zeigten sich artspezifische Wachstumsraten in verschiedenen Vermischungsmedien, wobei Moorwasser das Wachstum von Ostsee- und Moorisolaten signifikant stimulierte. Die Ostseearten sind dabei zusätzlich auf einen höheren Salzgehalt angewiesen, welcher über dem von Süßwasser liegt. Abschließend wiesen die Ergebnisse in **Publikation I und III** im Moorwasser eine stimulierende Wirkung auf das Wachstum der benthischen Diatomeen auf, welche durch die hohe Verfügbarkeit an organischen Kohlenstoffen in Moorwasser wahrscheinlich auf eine mixo- oder heterotrophe Lebensweise hindeuten.

Zusammengefasst zeigt diese Dissertation, dass Ostsee- als auch Moordiatomeen durch ihre hohe photo-physiologische Plastizität, euryhaline und eurytherme Toleranzen an die fluktuierenden abiotischen Variablen ihrer eigenen Habitate akklimatisiert sind. Die Daten weisen des Weiteren darauf hin, dass benthische Diatomeen im Rahmen von Renaturierungsmaßnahmen von Küstenmooren oder klimawandelbedingten Veränderungen ihrer Habitate bestehen.

1 General introduction

1.1 Coastal dynamics of the southern Baltic Sea

The Baltic Sea is the largest inland brackish-water basin located in northern Europe and represents one of the most unique ecosystems with pronounced abiotic gradients such as salinity and temperature (Snoeijs-Leijonmalm et al. 2017). Due to its enclosed characteristics, the Baltic Sea has relatively little water exchange with the adjacent saline North Sea to the southwest, and thus a water retention time of > 30 years (ESPON 2013). The high freshwater input via riverine discharge from a large catchment area of 1.74 million km² (HELCOM 2007) leads to horizontal salinity increases with decreasing latitude, from 2 S_A (absolute salinity) in the Bothnian Bay to as high as 27 S_A in the Kattegat (Lehmann et al. 2002). A similar gradient is found for the mean sea surface temperature, due to the Baltic Sea location in the temperate zone, with its typical seasonal and diurnal variations, generally increasing from north to south (Siegel et al. 2006). In winter, the average water temperature is around the freezing point, with seasonal ice cover in the north, and rarely, ice formation in the south (Høyer & Karagali 2016). In summer, in the southern Baltic Sea (Arkona Basin), surface water temperatures reach approximately 20 °C (Siegel et al. 2006).

The shallow coastal Baltic Sea is particularly subject to strong meteorological dynamics including wind and precipitation, which are amplified by fluctuating water-flow velocities along the shore. These dynamics continually shape the terrestrial coastline through erosion and land reclamation, which is mainly characterized by natural forests, wetlands, cliffs, and low-lying areas that are predominantly used for anthropogenic purposes (ESPON 2013). Due to the dependency of more than 15 million people inhabiting and using areas within 10 kilometers of the coast, the main priority of the population is the protection of especially low-lying coastal areas from coastal erosion and potential flooding (Sweitzer et al. 1996).

Coastal-protection measures along the 377 km of outer coast of the German federal state of Mecklenburg-Western Pomerania were effectively taken after a storm-surge event in 1872. The surge reached a maximum of 330 cm above sea level, flooding extensive areas with devastating consequences for coastal inhabitants (BSH 1872). Today, most of the Mecklenburg Baltic Sea outer coastline, including more than half of the low-lying floodplain areas, is protected from Baltic Sea water intrusion by 106 km of dunes and 45 km of sea dikes as physical defense barriers (Fröhle et al. 2011, StALU 2021). Furthermore, wooden groin systems were established in the shallow coastal waters to reduce horizontal sediment transport, thereby help stabilizing the coast and reduce coastal erosion. In recent decades, these coastal protection measures are increasingly threatened by the changing dynamics of the Baltic Sea water level as a consequence of global warming. Due to the increasing

emission of greenhouse gases such as carbon dioxide (CO₂) and methane (CH₄) (Olivier & Peters 2020), the mean global temperature has risen by 1 °C above preindustrial levels and will continue to rise (Foster & Rahmstorf 2011, IPCC 2018). The resulting glacial melting, especially in the Arctic (Stroeve et al. 2014, Dai et al. 2019), and thermal expansion of the Baltic Sea (Gräwe et al. 2019) have subsequently led to a sea-level rise in the southern Baltic Sea of 2 mm per year (PSMSL 2020). The sea-level increase is further enhanced by the glacio-isostatic subsidence of the southern Baltic Sea induced by glacial melting and a resulting weight shift in the tectonic plate, leading the south to subside (Johansson et al. 2004).

Additionally, global warming will lead to an increase in storm probability and storm intensity, resulting in more and more wind-driven episodic and sporadic storm-surge events (Vousdoukas et al. 2016). This will in turn affect the stability of the coastal-defense measures, leading to enhanced coastal erosion. In combination with the sea-level rise, the height of the storm surges is increasing (Gräwe & Burchard 2012). Over the past 60 years, the number of hours of 50 cm above mean sea level at the southern Baltic Sea coast has increased significantly, thereby exerting forces beyond the capacity of coastal-protection measures (Jurasinski et al. 2018). This can lead to major damage, especially to dunes and dikes, facilitating hydrodynamic and biogeochemical exchange processes across the marine-terrestrial interface and water intrusion into the hinterland, which were previously reduced or prevented.

Along the coast, especially low-lying areas, e.g., coastal fens, are vulnerable to flooding caused by increasing sea levels and damage to dunes and dikes (StALU 2010). Coastal fens are nutrient-rich, waterlogged wetlands where plant detritus and organic matter accumulate, forming peat soil, and are therefore often referred to as peatlands (Xu et al. 2018). The characteristic formation of peat is caused by rising groundwater levels and accumulation of organic material (Dahms 1991). Due to the water-saturated conditions, peatlands are lacking in oxygen with low redox potential, slowing the decomposition of the peat soil. These conditions result in storage of large amounts of carbon, making peatlands important carbon sinks (Gallego-Sala et al. 2018). Even though peatlands cover only ~3 % of the global terrestrial area (Xu et al. 2018), they have tremendous CO₂ storage potential and account for 30 % of the global soil carbon storage, more than that of all global trees (Parish et al. 2008).

Along the Mecklenburg Baltic Sea coast, approximately 40,000 ha of the 2,317,400 ha total area is covered by low-lying coastal peatlands (Jurasinski et al. 2018). Over 95 % of these peatlands are considered degraded (Tanneberger et al. 2021) as caused mainly by drainage due to peat mining and conversion to farmland (Xu et al. 2018). A lack of water supply in the peatland leads to oxygenation of the peat soil and increased aerobic microbial decomposition. Consequently, the associated release of previously bound CO₂ (Parish et al. 2008) accounts for 5 – 10 % of the global and 2.5 – 5 % of the German total atmospheric CO₂ emissions (Freibauer et al. 2009, Loisel et al. 2021). In relation to climate

change and increasing CO₂ concentrations in the atmosphere (Olivier & Peters 2020), pristine peatlands are becoming increasingly recognized as carbon sinks. Therefore, and as a natural and ecologically effective strategy, it is important to protect pristine and to rewet drained peatlands.

1.2 Renaturation of coastal peatlands and the change in coastal dynamics

Renaturation of coastal peatlands to recover the original state is performed more and more frequently (Vasander et al. 2003). Since the 1970s, renaturation of peatlands was mainly done to restore vegetation, increasing diversity and nature conservation. However, due to the impact of pristine peatlands on the global CO₂ balance and the direct impact on climate change, the motivation of renaturing peatlands has shifted toward increasing CO₂ storage (Schmatzler & Schmatzler 2010, Jurasinski et al. 2020). Globally and locally, renaturation of peatlands can increase CO₂ storage, thereby retaining CO₂ emissions, e.g., for Germany by 35 million t CO₂-eq ha⁻¹ y⁻¹ as estimated by the German Federal Office for Nature Conservation (BfN 2010).

The fundamental principle of renaturation of peatlands includes raising and stabilizing the water table to permanently water-saturate the soil/peat, whereby many variables such as the local climate and vegetation of the catchment area must be considered. For coastal peatlands, the return to a pristine system often includes dismantling or complete removal of dunes and dikes to facilitate exchange processes with the adjacent ecosystem, the Baltic Sea. (Miegel et al. 2016, Seifert & Herrmann 2020). As a result, the complex hydrological processes in the peatland will change, especially along the interface (Schreiber et al. 2021).

The consequences of renaturation of coastal peatlands and the emerging exchange processes along the marine-terrestrial gradient are so far scarcely investigated, with few studies focusing on specific issues, e.g., the impact of coastal erosion or changes in physical properties of the peat soil (Lampe et al. 2011, Urbanová et al. 2012, Lamers et al. 2015, Jurasinski et al. 2020). Due to the importance of understanding the underlying processes along the marine-terrestrial gradient, the number of collaborative scientific studies on hydrological and geochemical processes in renatured peatlands along the German Baltic Sea, e.g., in Baltic TRANSCOAST (Jurasinski et al. 2018, Janssen et al. 2019) and WETSCAPES (Jurasinski et al. 2020), has increased. Nevertheless, these processes will affect not only the physical properties of the peatland and the coastal Baltic Sea but also biological processes in organisms inhabiting these ecosystems.

1.3 Biota along the Baltic Sea coastal peatland ecocline

Coastal peatlands and the coastal Baltic Sea are complex, biodiverse ecosystems (Ojaveer et al. 2010, Minayeva et al. 2017). As a result of dune or dike removal during the renaturation process, these two

ecosystems are sporadically or episodically connected along the ecocline, which can influence the organisms in these habitats. In some cases, such as in the coastal peatland Hütelmoor on the German Baltic Sea coast, a peatland can also influence the shallow Baltic Sea before the removal of the separating structure, e.g., via submarine ground water discharge (SGD) from a peat layer extending into the sea (Racasa et al. 2021). Therefore, it is important to understand the exchange processes between the respective ecosystems in order to create optimal hydrological conditions for maintaining diversity and the respective ecological functions (Lamers et al. 2002). Peatlands, in particular, contain uniquely adapted species with specific tolerances to hydrological and physical abiotic variations in e.g., salinity (Tiner 2018, Batistel et al. 2022). Of these variations, however, mainly higher plants are studied, as these represent a core component of the formation of peat soil and are therefore heavily involved in the carbon cycle (Zeh et al. 2020, Batistel et al. 2021). Nevertheless, smaller organisms also strongly contribute to this cycle, including the microphytobenthos, a phototrophic biofilm, found along the benthos of both these habitats (Barranguet et al. 1997). As with estuaries, the developing exchange processes between the Baltic Sea and coastal peatlands could influence the biological activity of these benthic communities (Leitão et al. 2015). And while the basic functions of the microphytobenthos in the Baltic Sea are known, no studies have examined this community in peatlands or in connection to peatland renaturation.

1.3.1 The microphytobenthos

The microphytobenthos is one of the most ecologically significant microbial communities forming biofilms in the photic zone of marine, brackish, and freshwater habitats, and is often recognized as a brownish layer on the surface of benthic substrates (Woelfel et al. 2007, Hope et al. 2020). These phototrophic communities include green algae, cyanobacteria, and dinoflagellates, and are often dominated by pennate diatoms (Colijn & De Jonge 1984, Hillebrand & Sommer 1997, Paterson & Hagerthey 2001), which contribute around 20 – 25 % of the marine primary production (Falkowski et al. 1998, Field et al. 1998, Cahoon 1999, Kromkamp & Forster 2003). The characteristic formation of these biofilms can be ascribed to excretion of sticky exopolymeric substances (EPS) as a result of carbon fixation of, e.g., diatoms, which via the attachment of their cells to the sediment grains stabilize the sediment surface (Goto et al. 2001, De Brouwer et al. 2005, Beninger et al. 2018). Beside the strong influence on the oxygen and carbon cycles, microphytobenthic organisms depend on inorganic nutrients to fuel their photosynthetic metabolism, whereby they also contribute significantly to the nitrogen, phosphorus, and silica cycles (Barranguet et al. 1997, Sundbäck et al. 2000, McGlathery et al. 2004, Wilhelm et al. 2006, Leynaert et al. 2011, Tréguer & De La Rocha 2013). Inorganic nutrients can be acquired from the water column, but as benthic organisms, the microphytobenthos also benefits

from the typically nutrient-rich pore water in the sediment, making them important controllers of vertical nutrient fluxes at the sediment-water interface (Admiraal 1984, Risgaard-Petersen et al. 1994). Moreover, as primary producers, the microphytobenthos is important in the aquatic food web, constituting a food source for filter-feeders and feeding the microbial loop via dissolved organic matter (DOM) (Middelburg et al. 2000, Sommer et al. 2002, Iñora et al. 2003).

The microphytobenthos is widely distributed in the shallow waters of the Baltic Sea, where the biomass and productivity of these communities are strongly dependent on multifactorial spatio-temporal environmental gradients (Kuriyama et al. 2021). Especially in protected shallow-water areas, such as the “Boiensdorfer Werder” along the Mecklenburg coast, where wind- and wave-induced mixing of the upper sediment is strongly reduced, highly productive microphytobenthic biofilms form (Karsten et al. 2021). In exposed locations along the southern Baltic Sea such as in front of the Hütelmoor, where strong winds and currents characterize the benthos, high primary production was also observed (Kuriyama et al. 2021). These authors further showed that microphytobenthic biomass increased significantly at depths of up to 6 m in the exposed location, due to decreasing physical forces, with smaller diatoms dominating in the shallower depths, preferentially occurring in interstices of sediment grains rather than forming microphytobenthic biofilms (Kuriyama et al. 2021). While these pioneer studies have demonstrated the key role of the microphytobenthos as primary producers with focus on benthic diatoms in the southern Baltic Sea, studies on microphytobenthic diatoms in adjacent aquatic systems such as coastal peatlands are lacking. As shown in the study by Gerbersdorf et al. (2005), which found high rates of microphytobenthic primary production in strongly terrestrially influenced aquatic systems such as the Kurr Bight in a Baltic Sea lagoon system, microphytobenthic primary production, specifically of the dominant diatoms, in peatlands must not be neglected. Since the study by Kuriyama et al. (2021) indicated that different diatoms occur in different environmental conditions, studies of species-specific diatom responses to environmental conditions should be a priority.

1.3.2 Diatoms: the dominant group in the microphytobenthos

Diatoms are phototrophic unicellular eukaryotic microalgae belonging to the class Bacillariophyceae. They are generally cosmopolitan (Vanormelingen et al. 2007) and occur in freshwater, brackish, and marine habitats as well as terrestrial areas (e.g., Pfister et al. 2017, Pandey et al. 2018). Their name is derived from the Greek word *diatomos* meaning “cut in half”, in reference to their two-part cell wall, the frustule (Armbrust 2009). The frustule, made from silica, is divided into two interlocking parts, called valves – the slightly larger epitheca and the hypotheca. The valves of each diatom species form different shapes and structures, which are used as the main guide for taxonomic classification. The two major differentiations of diatoms are based on their symmetry: centric diatoms (Centrales) and

pennate diatoms (Pennales). Centric diatoms are radially symmetrical and generally planktonic, while pennate diatoms are bilaterally symmetrical and typically dominate the microphytobenthos (Kooistra et al. 2007). Differences in the valve structure are used to define diatom taxa; for instance, the so-called fibulae, internal silica bars stabilizing the raphe canal, are specific to the pennate genus *Nitzschia*. These variations also include, for example, pores in varying shapes for exchange purposes, e.g., for excretion of EPS resulting from carbon fixation (Goto et al. 1999, Moerdijk-Poortvliet et al. 2018). A feature exclusive to pennate diatoms is the raphe, a small slit running lengthwise along the valve. This peculiarity enables so-called raphid diatoms to excrete EPS through the raphe, allowing for vertical and horizontal gliding locomotion in and on the sediment (Hopkins 1964, Blommaert et al. 2018). This allows the diatom to respond to external stressors such as light availability (Perkins et al. 2010). Depending on the species, pennate diatoms developed raphes on two valves (biraphid), on one valve (monoraphid), or no raphe at all (araphid), with movement generally found in biraphid species (Pringle 1985).

Diatoms have a unique life cycle with size-dependent facultative sexual and vegetative reproduction (Vyverman 2004). During the more frequent and prolonged vegetative phase, diatoms reproduce by mitotic division, with the epitheca and the hypotheca separating, each now forming the epitheca of the daughter generation (Round 1972). The formation of a new, smaller hypotheca causes a reduction in mean cell size with every reproduction cycle, and hence a continuous decrease in cell size in a population. Therefore, the vegetative cycle is size-limited and thus leads to incapability of further reproduction. Cell size can be restored to its maximum by the formation of an auxospore during sexual reproduction (Davidovich & Bates 1998), which can also function as a resting spore under unfavorable conditions such as nutrient or light limitation (McQuoid & Hobson 1996). Thereby, sexual reproduction leads to larger cells and thus enables growth and ultimately the survival of a population (Drebes 1977, Vyverman 2004).

Fossil records indicated that diatoms have been populating marine systems for 190 million years (Sims et al. 2006) and became notable organisms in aquatic habitats about 100 million years ago (Armbrust 2009). The success of diatoms is often attributed to their evolutionary history, as they are well adapted to respond to environmental variations (Falkowski et al. 2004, Kooistra et al. 2007). According to current understanding, diatoms are eukaryotic chimeras (Kroth et al. 2008, Prihoda et al. 2012) that derived from secondary endosymbiosis, whereby a heterotrophic eukaryotic cell engulfed a photosynthetic eukaryotic cell with a red-algal origin (Bowler et al. 2008). The incorporated endosymbiont eventually developed into a plastid, maintaining a small genome (Armbrust et al. 2004). The ensuing complex gene transfer between the nucleus and plastid has resulted in genomic differences in diatoms, as found in *Thalassiosira pseudonana* Hasle & Heimdal, with almost half of the

diatom proteins showing similar alignment scores to their closest homologs in plant, red algal, and animal genomes (Armbrust et al. 2004). Studies of the intracellular bacteria Chlamydiae, which are closely linked to primary endosymbiosis, suggest that green algae also contributed to the genes acquired in diatoms (Becker et al. 2008, Armbrust 2009).

1.4 Consequences of peatland renaturation to the diatom habitat

Renaturation of coastal peatlands and facilitation of previously prevented exchange processes across the coastal ecocline are expected to strongly influence the respective habitats of peatland and Baltic Sea diatoms. Especially, flooding caused by increasing storm-surge events (Vousdoukas et al. 2016) will facilitate the exchange of water between the Baltic Sea and the coastal peatlands, including transportation of organisms into the opposing environment that were originally separated, with variations of long- and short-term exposure to abiotic variations.

One of the most interesting features of water exchange between coastal peatlands and the brackish Baltic Sea is the change in salinity. With Baltic Sea water-inflow events, peatland diatoms are subjected to higher salinities. Contrariwise, Baltic Sea diatoms will experience lower salinities when transported into the peatland, as with no permanent connection to the sea, the water will become diluted by the fresher peatland water and increased freshwater input from the catchment area. These differences in salinity cause osmotic stress, e.g., by reducing the water potential in diatoms with increasing salinity, causing cells to lose water and thereby affecting their physiology (Scholz & Liebezeit 2012b). High concentrations of salts such as Na^+ and Cl^- can also have a toxic effect on diatom metabolism (Hagemann 2011). However, diatoms are able to regulate the intracellular osmotic pressure in response to salinity via adjustment of osmotically active substances in the cytoplasm and vacuoles (Kirst 1990). As a rapid response to salt stress, diatoms adjust the ionic concentrations of, e.g., Na^+ , Cl^- and K^+ in the cell via ionic pumps, thereby counteracting the external osmotic pressure (Zimmermann 1978). Furthermore, diatoms accumulate highly soluble, low-molecular-weight organic osmolytes, so-called compatible solutes, in the cytoplasm to balance the osmotic pressure (Scholz & Liebezeit 2012b). While these compatible solutes are energetically expensive, causing a decrease in the rate of cell division, they efficiently help to protect and maintain the metabolic processes in diatoms (Dickson & Kirst 1987, Bisson & Kirst 1995, Clavero et al. 2000, Krell et al. 2007, Woelfel et al. 2014). In diatoms, the osmoprotectant is most often the amino acid proline (Clavero et al. 2000).

In general, shallow-water benthic diatoms such as those from the Baltic Sea or estuaries have proven to be well acclimated to salinity changes, with wide growth tolerances that often exceed the salinity ranges typical of their habitat (Trobajo et al. 2011, García et al. 2012, Woelfel et al. 2014). While salinity has been shown to influence the morphology of diatoms, complicating morphological identification

due to variations in valve length or characteristic structures, it is not clear if the intracellular-regulation processes or the morphological changes of the diatom are the determining factor for different ecophysiological responses to salinity (Trobajo et al. 2011). Nevertheless, while the ecophysiology of benthic diatoms is affected by salinity changes, only one study has provided valuable insight into the wide salinity tolerance of Baltic Sea benthic diatoms, indicating growth in freshwater, brackish, and marine conditions in three diatom strains (Woelfel et al. 2014). So far, no studies have treated the ecophysiological response of peatland benthic diatoms to salinity changes, which is especially relevant in the context of renaturation of coastal peatlands and the resulting intermixing processes of two ecosystems with differences in salinity.

Transportation of diatoms into the respective new habitat is also followed by changes in water-surface temperature. Due to the different physical properties of the Baltic Sea and the peatlands, such as water depth and water circulation, peatlands along the German Baltic Sea coast have a much wider annual range of water temperatures, with ice cover in winter and summer surface temperatures around 30 °C compared to the Baltic Sea (personal observation), which may therefore be reflected in the diatom ecophysiological responses. Almost all biochemical reactions and metabolic rates in organisms are accelerated by increasing temperature (Brown et al. 2004), as confirmed for six phytoplankton diatom species in a study by Montagnes & Franklin (2001), which showed a linear increase in growth rates with increasing temperature from 10 to 24 °C. However, studies on benthic diatoms from the Ems-Dollard estuary as well as the coastal Baltic Sea, investigating growth rates over a temperature range of 5 and 30 °C (Admiraal 1977) and 7 to 27 °C respectively (Woelfel et al. 2014), found that growth rates decreased after reaching optimum growth temperature, pointing to ecophysiological limits in response to temperature. A similar pattern was observed for the photosynthetic activity of temperate diatom-dominated benthic communities, with increasing photosynthetic rates under rising temperatures and a subsequent decrease after reaching an optimum (Hancke & Glud 2004). As with any enzymatic activity, photosynthesis is dependent on temperature, including, e.g., the carbon-fixing enzyme Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo). For instance, this enzyme loses specificity to CO₂ under increasing temperature and binding O₂ instead, thereby decreasing photosynthesis rates (Salvucci & Crafts-Brandner 2004, Tcherkez et al., 2006, Ras et al. 2013). This is especially relevant for the carbon-fixation capacity of diatoms, in particular in the peatlands, as they strongly contribute to carbon fluxes (Armbrust 2009). While the role of microphytobenthic diatoms in peatlands has yet to be studied, initial studies of phototrophic microbes in temperate peatlands indicated that they account for around 10 % of the total carbon uptake (Hamard et al. 2021) and are therefore crucial to understand the consequences of the underlying processes of the renaturation of peatlands in the context of the global carbon cycle.

Overall, marine diatoms are important primary producers and are responsible for around 40 % of the global carbon fixation (Nelson et al. 1995). To maintain their photosynthetic efficiency, they are therefore strongly dependent on light availability under water (Gleich et al. 2020), which is widely variable in the Baltic Sea and coastal peatlands. In response to the light conditions in their habitat, diatoms have developed strategies to regulate and optimize their photosynthetic potential. Despite the shallower depth, high turbidity in the peatland strongly attenuates the incident light, leaving benthic diatoms to live under low-light conditions, where photosynthetic activity is expected to be decreased. Under such low-light conditions, diatoms have been found to increase light-harvesting capacity by increasing their chlorophyll content (Anning et al. 2000) or altering the pigment composition in the chloroplast (Nagao et al. 2018). Besides chlorophyll *a* and *c*, light-harvesting pigments in diatoms also include the accessory pigment fucoxanthin, which absorbs more in the green range of photosynthetically active radiation (PAR), enhancing the light-harvesting capability of diatoms (Scarsini et al. 2019). In contrast, dynamics in the Baltic Sea can transport benthic diatoms toward the shoreline, with sea surface-like high light availability. In response to oversaturating PAR, diatoms have developed photoprotective strategies to counteract a decrease in photosynthetic activity or permanent photodamage. Excess light can lead to the formation of a triplet chlorophyll reacting with oxygen, forming photo-damaging reactive oxygen species (ROS) (Apel & Hirt 2004). To prevent the formation of ROS and potential damage to the photosystem II (PSII) and the antenna pigments (Krieger-Liszkay 2005), diatoms can dissipate excess energy as heat, a process termed non-photochemical quenching (NPQ) (Goss & Lepetit 2015). Diatoms contain two carotins, diadinoxanthin and diatoxanthin, which are crucial in the photoprotection of the light-harvesting complex under high-light exposure via the reversible epoxidation of diadinoxanthin to diatoxanthin and the resulting effective quenching (Goss & Jakob 2010, Blommaert et al. 2021). On the other hand, physical dynamics in the Baltic Sea and the peatland can also lead to cell burial in the sediment, thus shading them and leading to a deficit in PAR for photosynthetic activity. Nevertheless, some diatoms can also use a mixo- or heterotrophic metabolism, using organic compounds to survive, while resuming their function as phototrophs under improving PAR conditions (Villanova et al. 2017). Both the phototrophic and mixo-/heterotrophic metabolism may be enhanced with the exchange processes between the Baltic Sea and coastal peatlands. Peatlands are often rich in inorganic nutrients from the degraded peat soil and nutrient input via excess fertilizer runoff from farms in the catchment area (Koskinen et al. 2017), providing inorganic resources for phototrophic metabolisms. Furthermore, peatlands form from half-decomposed plant material due to the low oxidation potential and are therefore rich in dissolved organic carbon (Xu et al. 2018) thereby providing substrate for the mixo-/heterotrophic metabolism.

Despite the possible impact of the renaturation process and subsequent exchange processes on the metabolism of benthic diatoms, this has never been investigated.

1.5 Aim of the study and hypotheses

With the climate change-driven sea-level rise and increasing storm-surge events, coastal reconstruction and facilitation of exchange processes between the shallow Baltic Sea and coastal terrestrial areas will be inevitable. In relation to the sustainable reduction of greenhouse gases in the atmosphere, renaturation of coastal peatlands must be a priority, as they constitute approximately 25 % (600 gt) of the global soil carbon stock (Yu et al. 2010, Loisel et al. 2021). Despite the tremendous effects of microphytobenthic diatoms on the global carbon cycle as primary producers, contributing up to 20 – 25 % of global primary production and thus carbon fixation (Field et al. 1998, Sarthou et al. 2005, Serôdio & Lavaud 2020), the consequences to the microphytobenthos of, especially, water-exchange processes between coastal and terrestrial areas have not yet been included in the consideration of peatland developments.

On a geographic scale, the role of microphytobenthic communities in shallow coastal waters has been studied at many sites for decades (e.g., Sundbäck & Granéli 1988, Serôdio et al. 2012, Hope et al. 2020), with considerable focus on photosynthetic activity (Colijn & De Jonge 1984, MacIntyre et al. 1996, Longphuir et al. 2007). While few studies on the Baltic Sea microphytobenthos exist (e.g., Gerbersdorf et al. 2005, Karsten et al. 2021, Kuriyama et al. 2021), investigations on primary production and growth on a species-specific level of the dominant diatoms in their respective habitats are rare (Woelfel et al. 2014). Studies on microphytobenthic primary production in peatlands are completely lacking. Thus, there are no indications of the ecophysiological responses of benthic diatoms in the shallow Baltic Sea and adjacent peatlands to the changing abiotic variables in their habitat, as well as to subsequent abiotic changes because of the renaturation processes and the facilitation of storm-surge induced exchange processes between the Baltic Sea and the coastal peatlands.

Therefore, the objective of this dissertation was to gain insight into and expand the knowledge of ecophysiological responses concerning photosynthesis, respiration, and growth of benthic diatoms isolated from the Baltic Sea and for the first time adjacent coastal peatlands to the predominant abiotic variables of light (**Chapter 3.1**), temperature (**Chapters 3.1 and 3.2.2**), and salinity (**Chapter 3.2.1**). Furthermore, the impacts on growth (**Chapters 3.2.3, 3.2.4, and 3.2.6**) and photosynthetic activity (**Chapter 3.2.5**) of benthic Baltic Sea and peatland diatoms in response to simulated intermixing processes, as a reflection of the renaturation of coastal peatland and affected abiotic variables, were investigated.

In view of the information provided in the general introduction and the aim of this dissertation, four hypotheses were developed and are addressed in the synoptic discussion (**Chapter 3**).

Hypothesis 1:

Baltic Sea and peatland benthic diatoms show photosynthetic and respiratory optima in response to increasing photon flux densities (PFD) and temperature, reflecting the predominant annual fluctuations in their respective habitats.

In **publications I** and **II**, the evolution of photosynthetic and respiratory oxygen of established unialgal diatom strains isolated from the shallow coastal Baltic Sea and coastal peatlands was determined. Photosynthesis-irradiance (PI-) curves under increasing PFD of 0 – 1600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 20 °C were created, using dipping probe oxygen sensors. Under photosynthetically saturated light conditions, the evolution of photosynthetic and respiratory oxygen of the diatom strains in response to increasing temperatures from 5 – 40 °C was determined using the same methodological approach.

Hypothesis 2:

Baltic Sea and peatland benthic diatoms show growth optima in response to salinity and temperature, reflecting the predominant annual fluctuations in their respective habitats.

In **publication II**, growth of established unialgal diatom strains isolated from the shallow coastal Baltic Sea and coastal peatlands in response to salinity and temperature was investigated. Using *in-vivo* fluorimetry, specific growth rates of the diatoms grown in culture medium were determined and calculated during the exponential phase, at salinities ranging between 1 and 39 S_A and temperature ranges between 5 and 30 °C.

Hypothesis 3:

Increased proportions of peatland water during the intermixing of the two water bodies, the Baltic Sea and coastal peatlands, stimulate growth and photosynthesis of the benthic diatoms from both habitats, due to higher concentrations of nitrogen and phosphorus in the peatland water.

In **publication III**, the growth of established unialgal diatom strains isolated from the shallow coastal Baltic Sea and coastal peatlands in response to growth media with different proportions of peatland to Baltic Sea water, salinity adjustment and added nutrients was investigated. Ten growth media were developed, simulating the coastal intermixing processes between the Baltic Sea and the coastal peatlands. Using *in-vivo* fluorimetry, specific growth rates in response to the ten growth media were determined and calculated during the exponential phase. PI-curves under increasing PFD of 0 – 1600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 20 °C were created after 4 d of culturing in 50 % peatland + 50 % Baltic Sea

water and 100 % peatland water (15 S_A), using dipping probe oxygen sensors. Under photosynthetically saturated light conditions, the photosynthetic and respiratory oxygen evolution of the diatom strains in response to increasing temperatures from 5 – 40 °C was also determined. Additionally, specific growth rates in 18 growth media with increasing concentrations of nitrogen and phosphorus were investigated, using *in-vivo* fluorimetry (**Chapter 3.2.4**) and compared to diatom growth in the ten mixed media in **publication III**.

Hypothesis 4:

Peatland water stimulates mixo-/heterotrophic growth in diatoms.

In **publication III**, mixo-/heterotrophic growth was investigated in established unialgal strains isolated from the shallow coastal Baltic Sea and coastal peatlands and cultured in peatland and Baltic Sea water that differed widely in the content of dissolved organic compounds. Specific growth rates were determined using *in-vivo* fluorimetry. In **publication I**, diatom growth (as cell count) was determined after 10 d of growth in Baltic Sea and peatland water under complete darkness. Cell counts, using an inverted light microscope, were used as a proxy for growth.

2 Study sites and methodology

2.1 Study sites

Especially in the German federal state of Mecklenburg Pomerania, the renaturation of coastal peatlands will continue to play an important role in the coming years (Schiefelbein et al. 2011). Therefore, three coastal peatlands along the German Baltic Sea coast that are currently undergoing renaturation were selected to address the generated hypotheses. The study sites included the Hütelmoor peatland, the Polder Drammendorf and the Karrendorfer Meadows, which are in different temporal stages of renaturation (**Fig. 1**).

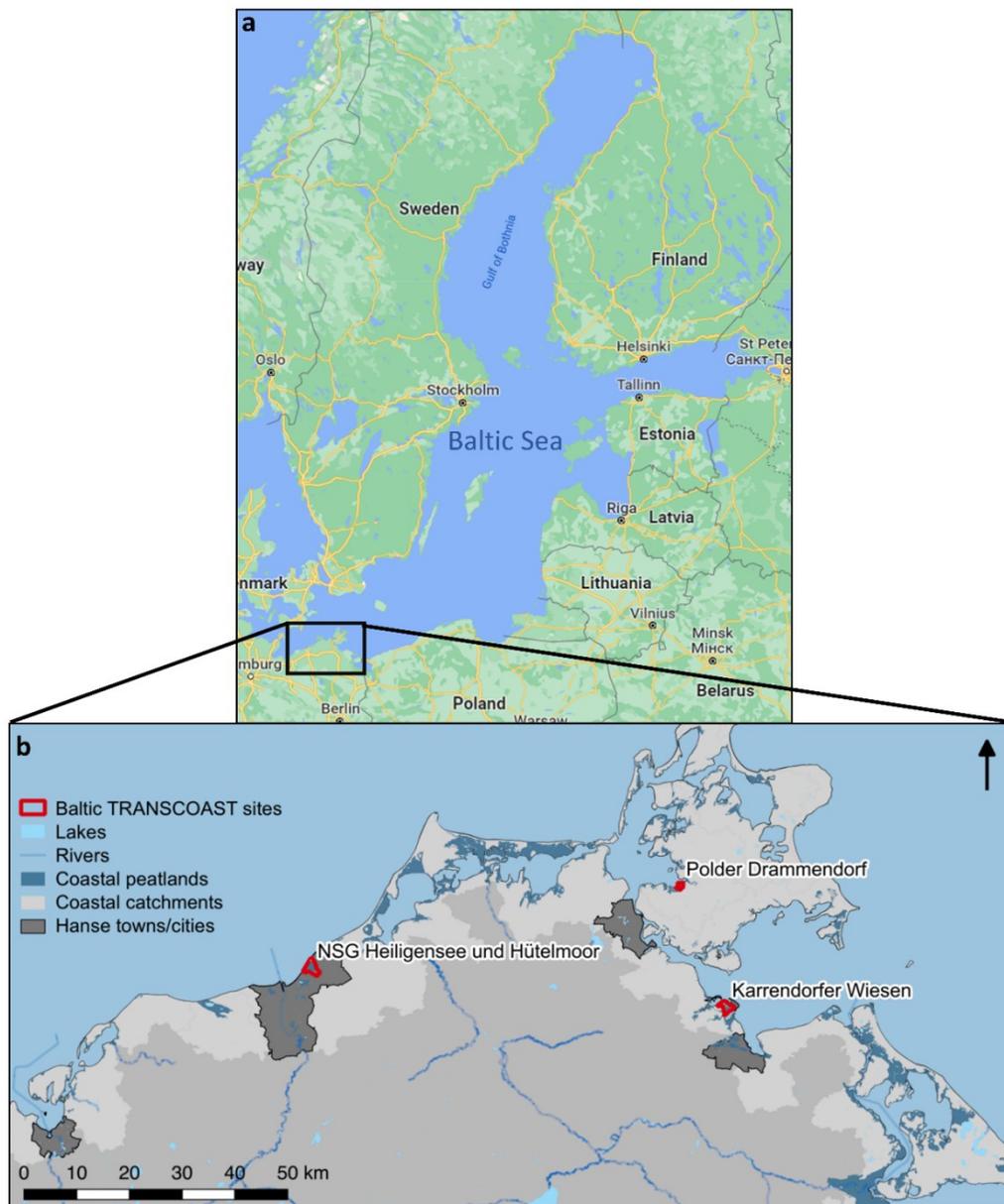


Figure 1: Overview of the locations of the sampling sites with **a)** the Baltic Sea coast (modified after Google Maps, 30.04.2022: Map data © 2022 Google, GeoBasis-DE/BKG, © 2009) and **b)** the three major sampling sites (modified after Janssen et al. 2019).

2.1.1 Heiligensee and Hütelmoor nature reserve

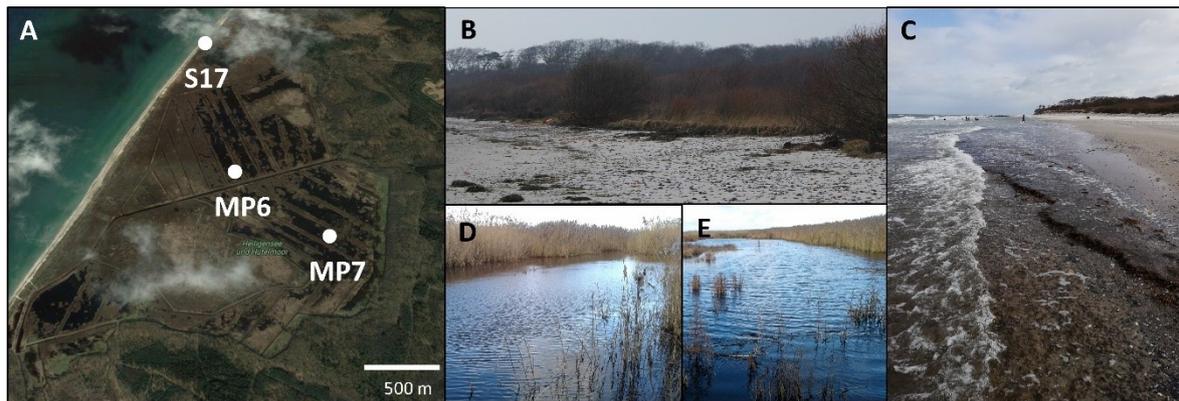


Figure 2 (A – E): Impressions of the Hütelmoor sampling area. **A)** Aerial overview from Google Maps (01.04.2022, Imagery © 2022 AeroWest, GeoBasis-DE/BKG, GeoContent, Maxar Technologies, Map data © 2022 Google, GeoBasis-DE/BKG, © 2009) with dots marking the sampling sites. Representative ground views showing: **B)** the dune breakthrough after the storm-surge event, **C)** Baltic Sea sampling site S17 with the exposed peat layer, **D)** overview of the peatland sampling site MP6, and **E)** overview of the peatland sampling site MP7.

The main study site was a coastal fen adjoining the Baltic Sea, called “Hütelmoor” (**Fig. 2 A – E**). The Hütelmoor is located in the “Heiligensee und Hütelmoor” nature reserve in Mecklenburg Pomerania, northeast of Rostock, and covers an area of around 3.5 km² (Miegel et al. 2017). Until 2019 the Baltic Sea and the Hütelmoor were separated by a dune that prevented continuous direct water exchange between the two ecosystems.

Since the early 2000s, the Hütelmoor has been undergoing a renaturation process, including removal of the separating dune by natural degradation, with no further maintenance. Additionally, the construction of a ground sill at the area water outlet allowed water retention in the lowland, thus reaching an average water level of ca. 40 cm (Miegel et al. 2017). Multiple storm-flood events, especially in 2006 (BSH 2006) and 2017 (BSH 2017) led to extensive erosion of the dune. The dune erosion was increased by the sediment transport northeastward due to strong currents and west winds along the shore (Lampe et al. 2011). While sediment transport along the Baltic Sea is often reduced by groin systems, as part of the restoration project the groins were not replaced when damaged, and in early 2022 were permanently removed in front of the dune opening.

Water in the peatland is mostly supplied by precipitation, groundwater, and the ditch system in the catchment area. Nevertheless, the sea-level rise of the Baltic Sea, such as during a storm-surge event in 2017 that reached 160 cm above sea level (BSH 2017), resulted in an inflow of Baltic Sea water into the peatland via the ground sill, increasing the salinity above freshwater levels (Miegel et al. 2017, Janssen & Racasa personal communication). On January 2nd, 2019, a storm surge with a sea-level increase of 183 cm above sea level led to a break in the northern dune and inflow of Baltic Sea water

directly into the Hütelmoor (BSH 2019). Electrical-conductivity measurements of the surface water of the peatland after the storm flood showed strong salinity changes associated with the flooding (Gutekunst et al. 2022). Thus, such storm-surge events with the accompanying flooding are also hypothesized to influence the hydrological and biogeochemical processes along the coastal Baltic Sea and are currently being investigated by the graduate college Baltic TRANSCOAST. The Hütelmoor area is especially interesting because of the unique feature of a peat layer that extends into the Baltic Sea below the beach (**Fig. 2 C**). The peat layer discharges submarine groundwater into the sea, thereby influencing the coastal Baltic Sea despite the dune separation (Racasa et al. 2021). The assumed changes in hydrological processes after the dune break are currently under further investigation (Jurasinski et al. 2018, Janssen et al. 2019).

2.1.2 Polder Drammendorf

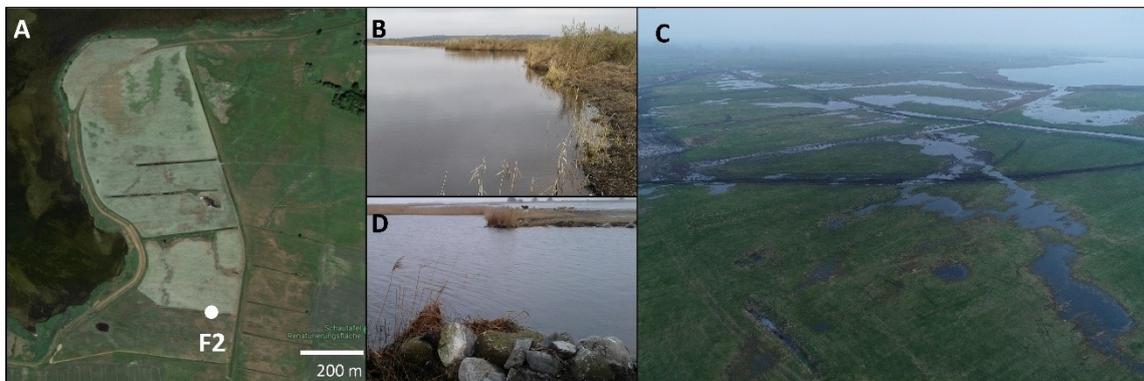


Figure 3 (A – D): Impressions of the Polder Drammendorf sampling area. **A)** Aerial view from Google Maps (01.04.2022, Imagery © 2022 GeoBasis-DE/BKG, GeoContent, Maxar Technologies, Map data © 2022 Google, GeoBasis-DE/BKG, © 2009); dot marks the sampling site. Representative ground views of **B)** the coastal area before the breach, **C)** the flooded area during the breach (image by Matthias Naumann), and **D)** the coastal area after the breach.

The Polder Drammendorf is a coastal fen situated east of the Hütelmoor on the Island of Rügen. Up to 2019, this area was separated from the Baltic Sea and more specifically from the adjacent Kubitzer Bodden via an artificial dike (**Fig. 3 A – D**). For more than 100 years, this terrestrial area, with an underlying peat layer and few trenches influenced by rain or higher groundwater levels, was used for agricultural purposes such as cattle and sheep pasture (Janssen et al. 2019). As part of its renaturation, an active dike removal took place in November 2019, creating an opening between the Kubitzer Bodden and the polder. In contrast to the Hütelmoor, which was originally rewetted by freshwater input from the catchment area and later influenced by Baltic Sea water, the polder was rewetted immediately by Baltic Sea water. Due to its low-lying nature, with a terrain of -0.6 to 3.0 m above

mean high tide, large areas were and will be permanently flooded with Baltic Sea water (**Fig. 3 C**) depending on the changing water level in the Kubitzer Bodden (Janssen et al. 2019).

2.1.3 Karrendorfer Meadows

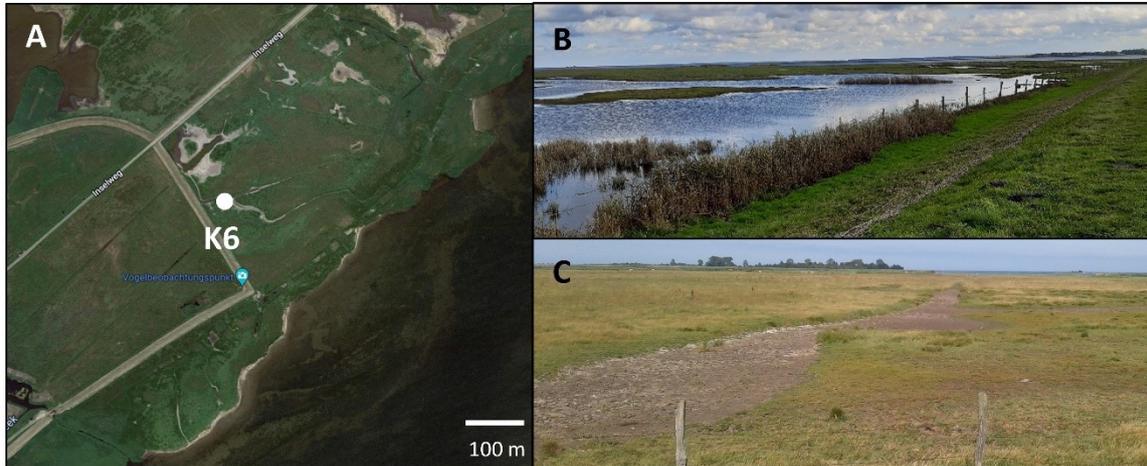


Figure 4 (A – C): Impressions of the Karrendorfer Meadows sampling area. **A)** Aerial view from Google Maps (01.04.2022, Imagery © CNES / Airbus, GeoBasis-DE/BKG, GeoContnet, Maxar Technologies, Map data © 2022 Google, GeoBasis-DE/BKG, © 2009); dot marks the sampling site. Representative ground views of **B)** the sampling area when flooded, and **C)** the sampling area when dry.

The Karrendorfer Meadows is a coastal fen located in the nature reserve "Insel Koos, Kooser See und Wampener Riff" along the shore of the Greifswalder Bodden (**Fig. 4 A – C**). In the 1850s, the meadows were separated from the Greifswalder Bodden, drained, and used for agricultural purposes (Janssen et al. 2019). In 1993, the area was rewetted after the deconstruction of the dike; it has been under restoration for almost 30 years (Seiberling et al. 2008). Due to its proximity to the Greifswalder Bodden and the low-lying terrain relative to the mean water level (Seifert & Herrmann 2020), water levels in the meadows vary over a short temporal scale, with higher potential for flooding in the autumn and winter (Mueller-Motzfeld et al. 2000) and for dry periods in summer (**Fig. 4 C**).

2.2 Methodology summary

The studies in this dissertation were conducted according to the schematic workflow illustrated in **Fig. 5** and are shortly summarized here. The shallow Baltic Sea adjacent to the Hütelmoor and the three coastal peatlands (**Fig. 1**) were sampled at approx. 30 cm water depth, using sediment cores which were later used as a basis for the diatom isolation process. Simultaneously, salinity, temperature, and PFD in the water column were measured *in situ* and water samples were taken for measurements of inorganic-nutrient concentrations (PO_4^{3-} , NO_3^- , NO_2^- and NH_4^+) and SAC_{254} as a proxy for dissolved organic matter (Nolde 2000), which were processed in the laboratory (**Publication I**).

In the laboratory, the upper 1 cm layer of the sediment cores was used for diatom isolation, following a similar approach to Stachura-Suchoples et al. (2016). The homogenized sediment was incubated for approx. two weeks in Guillard's f/2 medium (Guillard & Ryther 1962, Guillard 1975) enriched with metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 5 \text{H}_2\text{O}$; 10 g 100 mL⁻¹) at a final concentration of 0.6 mM. Single cells/small agglomerations of cells were then sorted with a glass pipette and diluted in culture medium in several steps, to obtain single cells. Clonal isolates were checked for unialgality, using a microscope (**Publication I**), and were cultured in Erlenmeyer flasks and regularly supplied with fresh medium until further use.

The isolates were identified to species in two steps: morphological identification using light and scanning electron microscopy, and molecular analysis. Diatom strains were morphologically identified via assessment of characteristic valve structures using light and scanning electron microscopy and comparison to taxonomic literature (**Publications I, II, and III**). As stated by Zimmermann et al. (2015), DNA (deoxyribonucleic acid) sequencing allows for the improvement of diatom identification via light microscopy. Therefore, molecular analyses of the *rbcL* gene (Ribulose biphosphate carboxylase large chain precursor), following the approach of Abarca et al. (2014), and the 18S V4 region (small subunit of ribosomal RNA with the largest variable region in eukaryotes), following the approach of Zimmermann et al. (2011) were performed. Sequences were then compared and deposited with the respective accession numbers in the data bank of the National Center for Biotechnology Information (NCBI) (**Publications I, II, and III**).

The ecophysiological responses to abiotic variables were determined in the laboratory. Rates of photosynthetic and respiratory oxygen evolution under increasing PFD and temperature were measured with oxygen optodes (Karsten et al. 2010), following the principle of dynamic luminescence quenching by molecular oxygen (John & Huber 2005; **publications I, II, and III**). The rates were related to the quantified chlorophyll *a* content, fitted with the respective photosynthesis model [Walsby (1997), Blanchard et al. (1996) and Yan & Hunt (1999)] and illustrated as PI-curves and temperature-dependent photosynthesis and respiration.

Specific growth rates in response to salinity, temperature, and different growth media (peatland water, Baltic Sea water, inorganic nutrients) were determined using *in-vivo* fluorometry, which is a robust non-invasive method particularly suitable for benthic diatoms (Karsten et al. 1996, Gustavs et al. 2009; **publications I, II, and III**). *In-vivo* chlorophyll *a* fluorescence, stimulated by blue light-emitting LEDs and detected as relative units, was used as a proxy for biomass and to calculate specific growth rates.

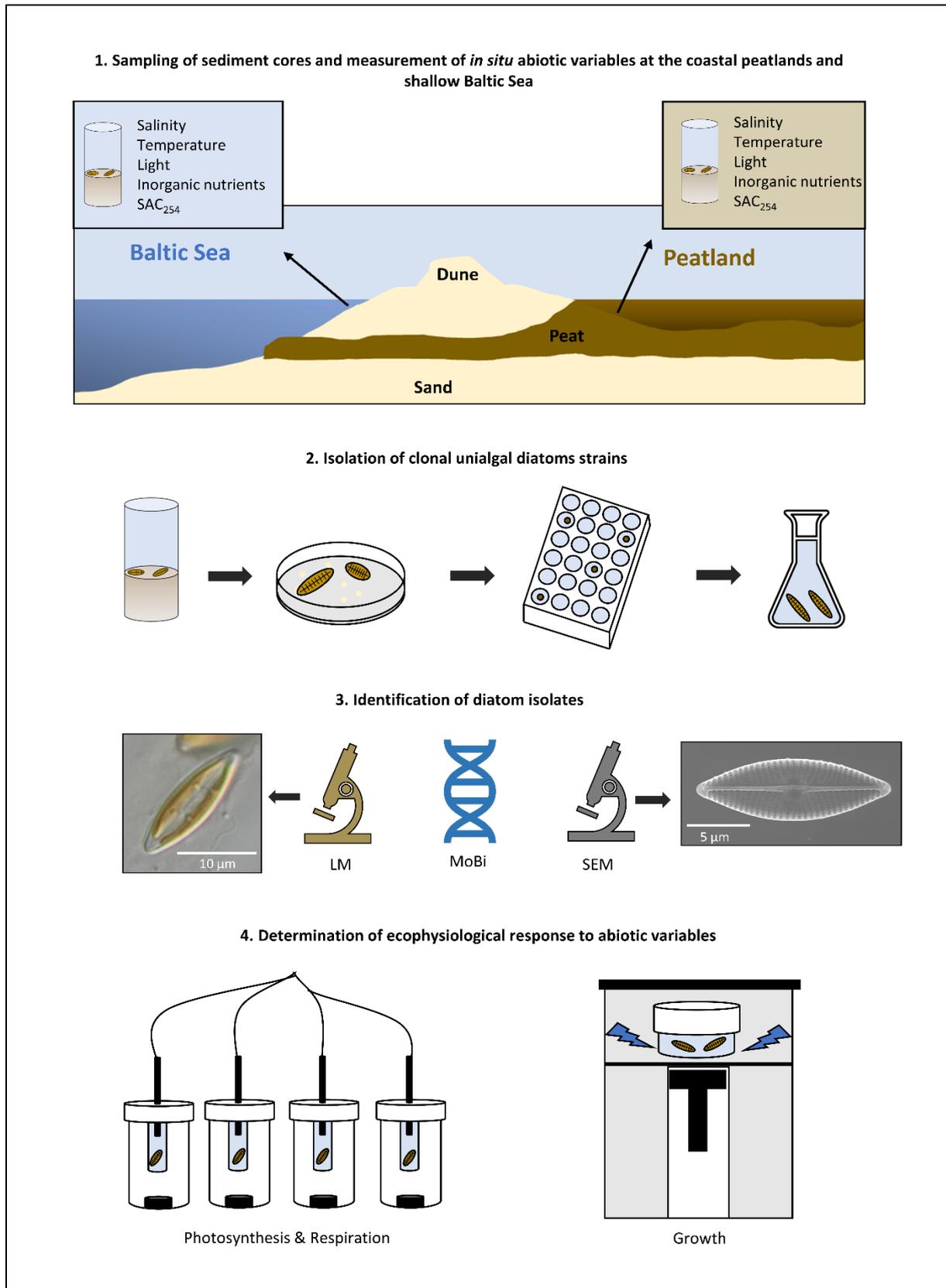


Figure 5: Schematic representation of the workflow to attain an insight into the ecophysiological responses of benthic diatom strains. The figure was created using Microsoft Office PowerPoint (2016) and the pictogram tool. LM = light microscopy, MoBi = molecular biology, SEM = scanning electron microscopy. The figure “growth” was adapted from Karsten et al. (1996).

As a result of the isolation processes, the following ten benthic diatom strains from the coastal Baltic Sea (**Fig. 6 A – I, M**) and three benthic diatom strains from coastal peatlands (**Fig. 6 J – L**) were investigated as representative taxa for their ecophysiological responses: *Actinocyclus octonarius* Ehrenberg, *Melosira moniliformis* (O.F.Müller) C. Agardh, *Halamphora* sp. 1 (Cleve) Levkov, *Nitzschia dubiiformis* Hustedt, *Nitzschia pusilla* Grunow, *Melosira nummuloides* C. Agardh, *Halamphora* sp. 2 (Cleve) Levkov, *Navicula perminuta* Grunow, *Navicula phyllepta* Kützing, *Nitzschia filiformis* (W. Sm.) F. Schütt, *Planothidium* sp. (st. 1) Round and Bukht., *Planothidium* sp. (st. 2) Round and Bukht., and *Hyalodiscus* cf. *scoticus* (Kützing) Grunow.

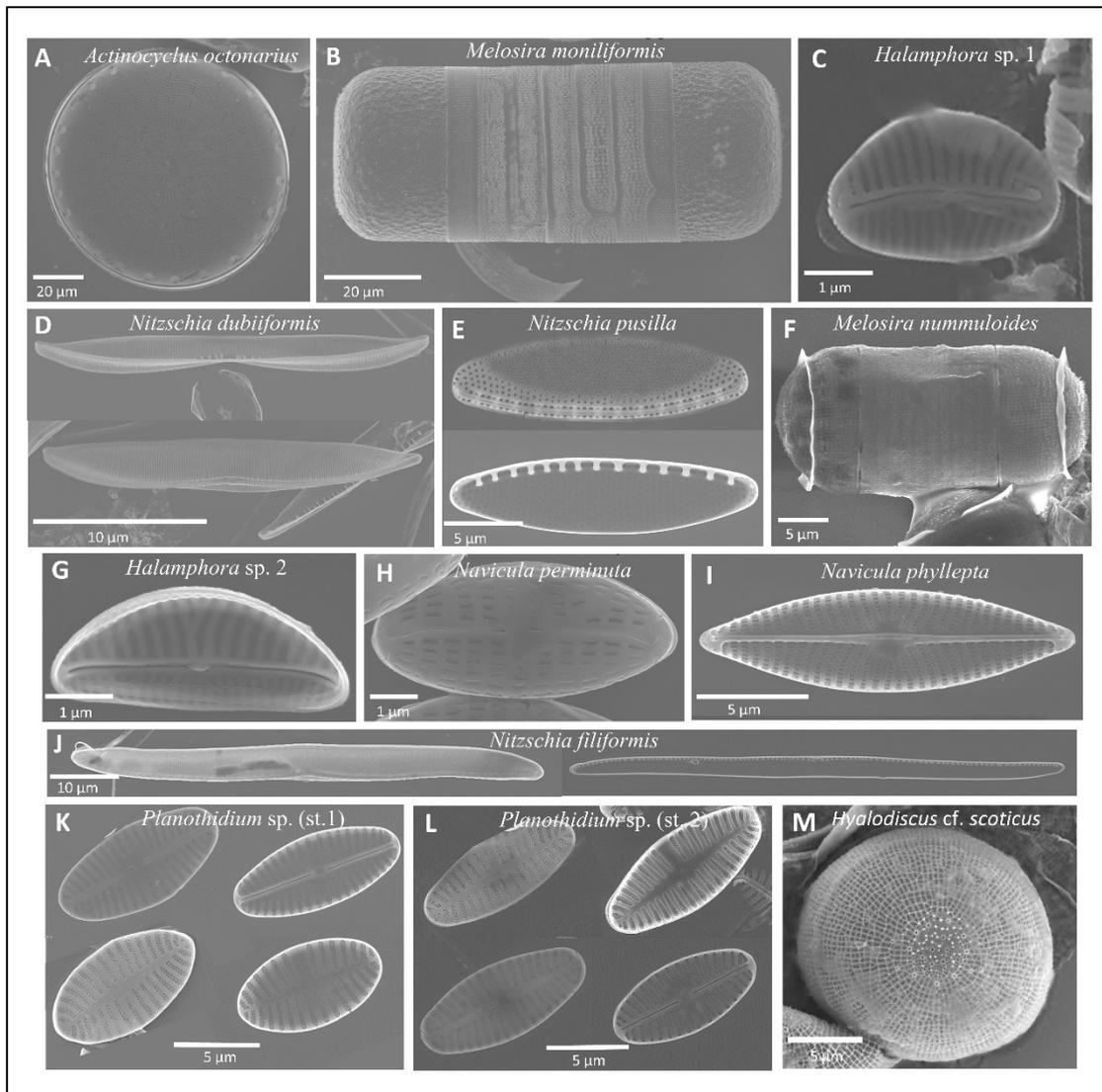


Figure 6 (A – M): Scanning electron microscopy images of benthic diatoms used in this study, with **A – I and M** originating from the coastal Baltic Sea and **J – L** originating from the three coastal peatlands. Images were obtained and modified from **publication I, II and III**.

3 Synoptic discussion

For over three decades, the diversity and functional roles of the microphytobenthos have attracted scientific interest (Colijn & De Jonge 1984, MacIntyre et al. 1996, Aberle-Malzahn 2004, Stal 2010, Hope et al. 2020). However, despite the growing knowledge of general functions of the microphytobenthos as, e.g., primary producers or food resources for filter-feeders (Hillebrand & Sommer 1997, Sundbäck et al. 2000, Méléder et al. 2018), concrete knowledge of the ecophysiology of the dominant benthic diatoms in the shallow Baltic Sea and adjacent peatlands is lacking (Woelfel et al. 2014). The data in this dissertation therefore contribute to the fundamental understanding of the ecophysiology of microphytobenthic diatoms, both as a function of naturally fluctuating abiotic variables in their habitats (**Publications I and II**), and in response to climate change-induced structural habitat changes along the Baltic Sea coastal peatland ecocline (**Publications I, II, and III**).

3.1 Photosynthesis and respiration of benthic diatoms

Benthic diatoms are often the dominant taxonomic group in the microphytobenthos (Colijn & De Jonge 1984, Cahoon 1999). As phototrophic organisms, photosynthesis is their key metabolic function, which is influenced by various environmental stressors (Scarsini et al. 2019). While the general photosynthetic responses of the Baltic Sea microphytobenthos to changing abiotic variables have been studied to some extent (Karsten et al. 2021, Kuriyama et al. 2021), species-specific responses of benthic diatoms along the Baltic Sea coast and of coastal peatland diatoms have been almost completely neglected. Until now, only one study by Woelfel et al. (2014) has investigated the photosynthetic response of three Baltic Sea benthic diatom species to changing abiotic factors, including light and temperature. The results showed high photo-physiological plasticity and eurythermal traits without loss of oxygen production under pre-identified suboptimal conditions of light (10 and 450 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; optimum 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and temperature (7 and 27 °C; optimum 24 °C). To significantly extend the knowledge of the photosynthetic activity of benthic diatoms under fluctuating light and temperature conditions, nine benthic Baltic Sea diatoms and, for the first time, three benthic peatland diatoms were studied in **publications I and II**.

3.1.1 Photosynthesis and respiration of Baltic Sea benthic diatoms

Publications I and II illustrate the high photo-physiological plasticity of the Baltic Sea benthic diatom strains investigated. Below 70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, all strains were able to perform saturated species-specific photosynthesis, thus demonstrating high photosynthetic potential under low-light conditions. These observations are in accordance with the findings of Woelfel et al. (2014), who observed

saturated photosynthesis $< 62 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for three benthic diatom strains from the shallow Baltic Sea at similar temperatures. While the low-light-saturation points suggest low light requirements, the strong species-specific photosynthesis rates of the nine Baltic Sea strains over increasing PFD of up to $\sim 1600 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ with no or low photoinhibition, rather indicate acclimation to a range of light conditions, including low light (**Publications I and II**). Due to complex biochemical light-harvesting mechanisms, e.g., increasing the light-harvesting capacity by increasing the chlorophyll content (Anning et al. 2000) and NPQ as a response to high-light conditions (Lavaud et al. 2004, Goss & Lepetit 2015, Blommaert et al. 2021), several studies have demonstrated high photo-physiological plasticity in benthic diatoms (e.g., Serôdio et al. 2006, Petrou et al. 2011, Woelfel et al. 2014).

Low-light acclimation is an important feature for the phototrophic diatoms living on the benthos of the Baltic Sea. Due to light attenuation in water, whereby incident PAR is decreased with increasing water depth, the benthos experiences lower light conditions than the sea surface. This physical property can be accentuated by turbidity from increased suspended inorganic and organic particles in the water column (Kirk 1994). Additionally, meteorological conditions, including wind, waves, and currents along the shallow southern Baltic Sea induce erosive forces, sediment resuspension, and nearshore soft-bottom sediment transport (Lampe et al. 2011, Jurasinski et al. 2018), likely burying and shading diatom cells (Saburova & Polikarpov 2003). Diatoms have been reported to survive through long periods of darkness (Antia 1976), especially in habitats with extreme conditions of low light availability such as found in polar regions, by reducing their metabolic activity (Palmisano & Sullivan 1982) or formation of resting stages (McQuoid & Hobson 1996). Schaub et al. (2017) reported that the benthic diatom *Navicula cf. perminuta* Grunow catabolized the storage lipids triacylglycerol (TAG) in the cytoplasm and the free fatty acids (FFA), while maintaining phospho- and glycolipids, thereby leaving the photosynthetic membranes of the chloroplasts untouched and functional. This accords with a study of the diatom *Phaeodactylum tricorutum* Bohlin, which managed to maintain high photosynthetic capacity even in long periods of darkness, indicating its potential to acclimate to varying periods in the dark (Griffiths 1973).

In the Baltic Sea, the maximum seasonal PAR fluctuated between 389 and 2117 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Mecklenburg Bight, Woelfel et al. 2014), sufficient for saturated photosynthesis (**Publications I and II**). Nevertheless, exposure of benthic diatoms to high light conditions can also lead to photoinhibition in the chloroplast and a persistent decrease in photosynthetic activity (Demmig-Adams & Adams 2017). A decrease in photosynthetic oxygen evolution was observed for six of ten strains at PFDs of up to $\sim 1600 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (**Publications I and II**). As a result of excess energy in the pigments/antennae, oxidative stress causes the development of ROS and damages the photosynthetic

apparatus, specifically PSII (Apel & Hirt 2004, Choudhury et al. 2017). To reduce any potential damage, diatoms use short-term photoprotective strategies. As a reversible response to high-light conditions, the photoprotective pigment diatoxanthin is accumulated via epoxidation of the xanthophyll diadinoxanthin, and excess energy is dissipated as heat; this is termed non-photochemical quenching (Lavaud et al. 2004, Goss & Lepetit 2015, Blommaert et al. 2021), with a species-specific NPQ capacity (Petrou et al. 2011). Excess energy that cannot be dissipated or retained leads to photo-oxidation and subsequently inactivation of the pigments (Hajiboland 2014). Along with this biochemical response, raphid diatoms are also able to physically evade unfavorable light conditions by moving vertically and horizontally in the sediment via excretion of EPS, thus allowing a photophobic response to photogradients (Perkins et al. 2010, Cartaxana et al. 2011, Beninger et al. 2018).

Although the microphytobenthos is typically dominated by pennate diatoms (Serôdio & Lavaud 2020), three centric strains were isolated from the Baltic Sea benthos, as described in **publications I and II**: *Melosira moniliformis* (**Publication I**), *Actinocyclus octonarius* (**Publication I**), and *Melosira nummuloides* (**Publication II**). Centric species in microphytobenthic communities in the Baltic Sea have also been reported by Vilbaste et al. (2000). Simultaneously, the authors noted many centric diatom resting spores, which are regularly deposited in the sediment (Mitbavkar & Anil 2002). While in the current study, resting spores were not investigated and therefore not determined, centric diatoms in the samples may have resulted from germination of resting spores under optimum growth conditions during the isolation process. Although all three strains showed photoinhibition, there was a large difference in the light-saturation point of *Actinocyclus octonarius* (**Publication I**), which was almost four times higher than those of the two *Melosira* species (**Publications I and II**). While the latter have been reported in the microphytobenthos (McLean et al. 1981, Woelfel et al. 2014, Lora-Vilchis et al. 2018), *Actinocyclus octonarius* is found primarily in the pelagic zone (Ochoa et al. 2010). The higher light-saturation point in *Actinocyclus octonarius* (**Publication I**) may therefore be explained by its generally higher PFD exposure in the water column compared to benthic diatoms, and hence its higher PFD requirements for saturated photosynthesis. This suggests that this species may have germinated from a resting spore or been transported to the benthos due to the coastal wind- and wave-induced mixing of the shallow coastal area, as it has not been reported previously in microphytobenthic communities. Nevertheless, with respect to the predominant PFD in the southern Baltic Sea, the results described in **publications I and II** clearly indicate a strong acclimation of the diatom species to fluctuating light conditions, and thus to their respective habitats.

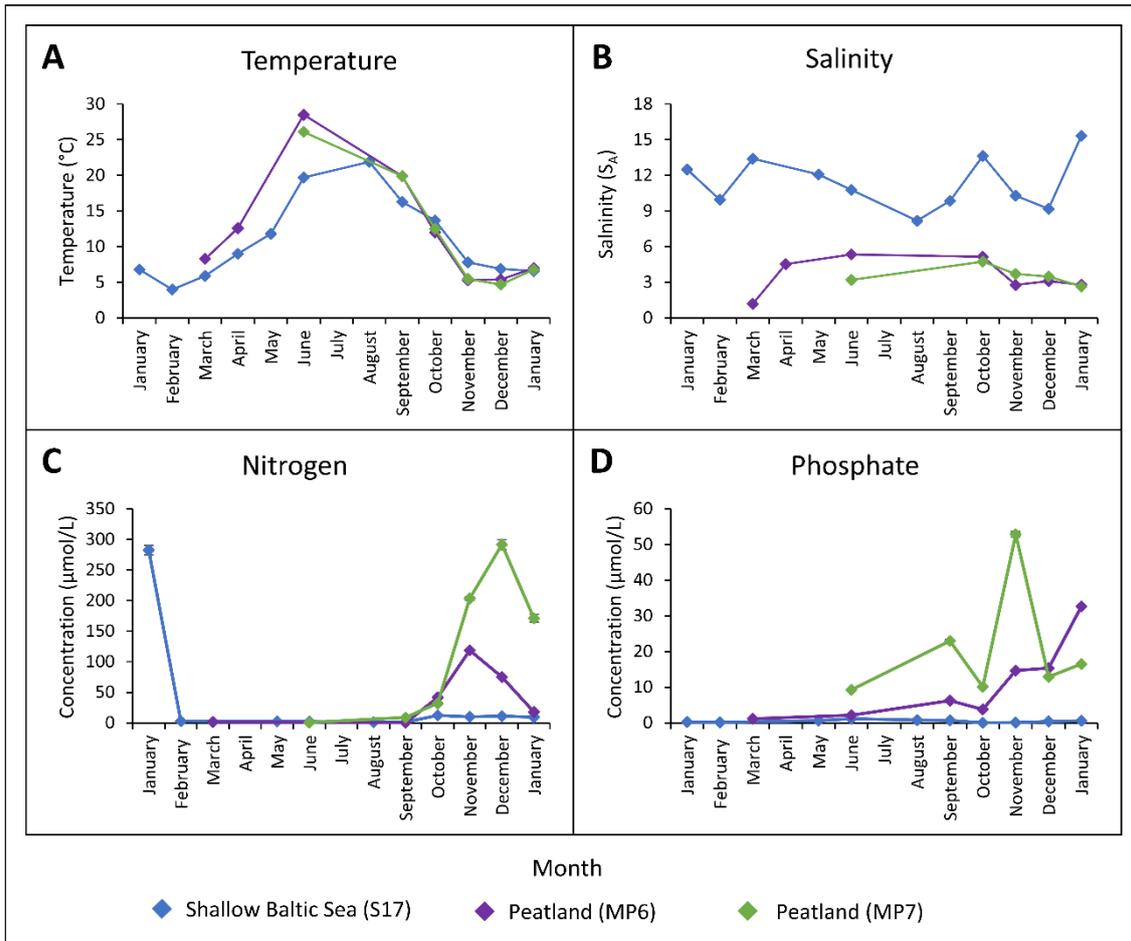


Figure 7 (A – D): Monthly abiotic variables from January 2019 to January 2020, measured approximately 30 cm below the surface water at the three Hütelmoor sampling sites S17, MP6, and MP7 (**Fig. 2**). Data represent mean values (**A – B**, $n = 1$; **C**, $n = 4$; **D**, $n = 2$). **A**) Temperature in °C, **B**) salinity in S_A , **C**) nitrogen (NH_4^+ , NO_3^- and NO_2^-) in $\mu\text{mol/L}$, and **D**) phosphate (measured as bioavailable orthophosphate) in $\mu\text{mol/L}$.

Besides the fluctuation in light conditions, the shallow Baltic Sea is also characterized by wide seasonal temperature changes (**Fig. 7 A**). Photosynthesis measurements in Baltic Sea diatom strains reported in **publications I and II** revealed a short-term eurythermal capability covering the annual temperature range of 4 – 22 °C measured at the respective sampling sites (**Fig. 7 A**). While photosynthetic activity is generally driven by light, it is also influenced by temperature due to the involvement of temperature-dependent enzymatic processes. As summarized by Gleich et al. (2020, and references therein), the light-dependent reactions of photosynthesis are little affected by temperature changes, while carbon assimilation during the Calvin-Benson-Bassham cycle by the key enzyme RuBisCO (Ribulose-1,5-bisphosphate carboxylase-oxygenase) is strongly driven by temperature (Falkowski & Raven 2013). Under saturated CO_2 conditions, increasing temperatures up to 30 °C increase the carboxylase activity of RuBisCO. However, temperatures above 30 °C can lead to the loss of CO_2 affinity by RuBisCO, binding O_2 instead and thereby reducing carbon assimilation (Salvucci & Crafts-Brandner 2004, Tcherkez et al.

2006, Ras et al. 2013). This process can result in decoupling of the photosynthetic and respiratory processes and in different optimum temperatures for photosynthesis and respiration. This was confirmed for the Baltic Sea diatom strains described in **publications I and II**, which showed optimum photosynthesis rates between 10 and 30 °C and optimum respiration rates between 10 and 40 °C. Similar species-specific results for photosynthetic and respiratory decoupling were observed in a study on benthic diatoms from temperate, northern temperate, and arctic zones, which found higher optimum temperatures for respiration than for photosynthesis (Hancke & Glud 2004).

3.1.2 Photosynthesis and respiration of peatland benthic diatoms

As discussed in **Chapter 3.1.1**, the ecophysiological responses of the microphytobenthos to habitat-specific abiotic changes in the shallow Baltic Sea have been investigated in only a few studies (Woelfel et al. 2014, Karsten et al. 2021, Kuriyama et al. 2021). So far, no studies have assessed the photosynthetic activity of the microphytobenthos or specific diatoms from coastal peatlands, despite the proximity of the two habitats. In the light of global climate change, the function of peatlands as carbon-storage sites has gained in importance (Parish et al. 2008, Leifelt & Menichetti 2018, Xu et al. 2018). Considering the significant contribution of the microphytobenthos to nutrient cycles and especially to the carbon and oxygen cycles (Armbrust 2009), these communities must be included in the consideration of the underlying processes. The photosynthesis activity of peatland diatoms was therefore addressed for the first time in **publication II**.

The results described in **publication II** confirmed that peatland diatoms show habitat-specific low-light acclimation, with photosynthetic light saturation points $< 75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and considerably lower light-compensation points $< 21 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. At the three coastal peatland sites, diatom strains were isolated from water depths of around 30 cm, which in the Baltic Sea would indicate higher light conditions. However, peatland surface water, in contrast to the Baltic Sea, contains large amounts of organic compounds, such as humic substances and organic particles (Davies-Colley & Vant 1987), contributing to absorption of incident light and adding to the physical light attenuation of water (Kirk 1994). Thus, in peatlands, PAR rapidly decreases in the water column, leading to low light conditions toward the benthos. For example, light measurements on a cloudy day in November 2019 at a depth of 30 cm from the water surface resulted in considerably lower PFD, between 7.8 ± 20.1 and $17.9 \pm 23.7 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the peatland sites of the Hütelmoor (**Fig. 2 A, D and E**), compared to between 33.9 ± 4.8 and $55.6 \pm 54.7 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the Baltic Sea sites (**Fig. 2 A and C**, unpublished, Prella and Mutinova). The high standard deviations of these measurements reflect the effect of additional factors on light availability, such as shading by macrophytes, cloud cover, and even infauna sediment bioturbation.

Despite the low light conditions along the benthos of the peatland, the three peatland diatom strains showed similar high photo-physiological plasticity to the Baltic Sea strains, with no significant photoinhibition up to $\sim 1600 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (**Publications I and II**). As the physical dynamics, e.g., wind-induced turbulence, in peatlands are rather small compared to the Baltic Sea, the potential for exposure of peatland benthic diatoms to high light conditions in shallow water depths is likely low. Due to the lack of studies in the area, the reason for the broad tolerances of the peatland diatoms to light is rather speculative. One possibility is the potential introduction of foreign diatom species to the peatlands by migrating waterfowl that use the area to rest (personal observation). However, *Nitzschia filiformis* has been recorded from a peatland canal (Mawarni et al. 2020), making this explanation rather unlikely. Moreover, as similarly high-light-tolerant benthic diatoms have been found in other low-light habitats such as the benthos of Potter Cove in the Antarctic (Prelle et al. in prep), this broad tolerance may be explained by the origin of the diatoms. It is hypothesized that diatoms evolved from a serial secondary endosymbiosis involving a heterotrophic flagellate ingesting a eukaryotic cell originating from red algae, thereby acquiring a range of features that diverge from higher plants, which may have aided in the success of the diatoms (Lopez et al. 2005, Armbrust 2009, Moustafa et al. 2009, Prihoda et al. 2012). Therefore, the broad photosynthetic tolerance may be the result of an adaptation rather than an acclimation to their habitat. However, these rough speculations clearly show that further studies are necessary to truly understand the ecophysiological tolerances and traits of the peatland diatoms.

In contrast to the Baltic Sea, peatlands are characterized as shallow, still, dark waterbodies. Due to the low albedo – the reflection capability – of the peatland water surface, especially in summer, solar energy is absorbed, leading to a rapid increase in the water-surface temperature of the peatlands, often exceeding 30 °C. Compared to the Baltic Sea surface water, which rarely exceeds 20 °C, it is assumed that peatland diatoms are eurythermal, with greater tolerance to higher temperatures than the Baltic Sea strains. However, **publication I** clearly illustrated similar tolerances for the Baltic Sea and peatland strains. Results in **publication II** thereby clearly illustrated for peatland diatoms to also exhibit eurythermal traits with moderate photosynthesis between 5 to 40 °C and decoupled respiratory and photosynthetic activity (**Publication II**). Therefore, Baltic Sea strains as well as peatland diatoms strains both cover the annual water temperature ranges of their respective and the adjacent habitat with no significant differences with respect to their origin (**Fig. 7 A**).

Overall, while benthic diatoms are very well acclimated to their respective habitat, strains from the peatland and the Baltic Sea show similarly high photo-physiological plasticity and eurythermal traits, with species-specific optima rather than habitat-specific optima, making them perfectly acclimated to annual abiotic variations that exceed the typical habitat conditions.

3.2 The consequences of intermixing between the Baltic Sea and coastal peatland water bodies on benthic diatom ecophysiology

In consequence of the increased frequency of extreme weather events and the resulting expected increase in exchange processes between the Baltic Sea and coastal peatlands, microphytobenthic diatoms will likely face changes in abiotic variables that may affect the ecophysiological responses of species from both habitats. Therefore, in **publications I, II, and III** the photosynthetic activity and growth response to variations in salinity, temperature, and growth media were investigated for the Baltic Sea and peatland strains.

3.2.1 Salinity effects on diatom growth

The Baltic Sea and peatland strains showed high tolerances to salinities of 1 to 39 S_A , with specific growth rates generally ranging between 0.5 and 1.6 μd^{-1} at species-specific salinity optima (**Publication II**), far exceeding the salinities in their respective habitats (**Fig. 7 B**). This is in accordance with the findings of Woelfel et al. (2014), who measured growth rates of three benthic diatom strains from the southern Baltic Sea at above the 20 % percentile between 1 and 50 S_A , and Scholz & Liebezeit (2012a), who investigated 25 benthic marine Wadden Sea diatom strains between 10 and 40 S_A . However, whereas Woelfel et al. (2014) suggested that the optimum growth of the strains was reflected in the habitat-typical salinity, **publication II** showed salinity optima much wider than the salinity ranges measured in the respective habitats (**Fig. 7 B**).

The southern Baltic Sea is affected primarily by fluctuating salinity conditions. Strong west winds can result in extreme saltwater-inflow events from the North Sea into the Baltic Sea, thereby increasing salinity (Schrum 2001). Salinity modeling of the southern Baltic Sea in 2014 with high resolution of the Warnow River estuary adjacent to the Hütelmoor illustrated the pronounced salinity variations within this small area along the shore, which range between 8 and 20 S_A (Lange et al. 2020). Furthermore, due to the formation of water pools along the beach caused by strong wave activity, which will eventually evaporate, especially in summer, diatoms are exposed to a range of salinities above 20 S_A . Especially high concentrations of salts such as Na^+ and Cl^- have toxic effects on the diatoms by inhibiting the metabolism due to dehydration of the cell (Hagemann 2011). In response to salt stress and the resulting influence on the osmotic potential in their cells, diatoms acclimate to various osmotic conditions. These acclimations include intracellular adjustment of the osmolarity by lowering the water potential in the cell via the accumulation of organic osmolytes, such as proline, betaines, or dimethylsulfoniopropionate (DMSP) (Bisson & Kirst 1995, Clavero et al. 2000, Woelfel et al. 2014), as well as adjustment of ionic concentrations of Na^+ , Cl^- and K^+ in the cell, thereby counteracting the external osmotic pressure (Zimmermann 1978).

Acclimation to the prevailing low salinities in the freshwater peatlands studied here is reflected in the growth tolerance of the peatland strains, with specific growth rates measured in salinities from 1 to 39 S_A at 0.5 to 1.6 μd^{-1} (**Fig. 7 B, Publication II**). The low salinities in the Hütelmoor are caused mainly by terrestrial freshwater runoff, precipitation, and groundwater input (Miegel et al. 2017). Nevertheless, measurements by colleagues at the study site indicated small sporadic increases in salinity caused by inflow of brackish Baltic Sea water via the ground sill (Janssen and Racasa, personal communications). These inflow events, however, have yet to raise salinity in the peatland to the level in the Baltic Sea. Nevertheless, measurements of electrical conductivity by Gutekunst et al. (2022) in the Hütelmoor immediately after the storm-surge event in January 2019 showed a salinity gradient directly from the Baltic Sea into the Hütelmoor, with conductivities at 11.6 and 22.4 $\mu S/cm$ ($\sim 6.7 - 13.7 S_A$) along the sea-land transition zone, 13.8 and 18 $\mu S/cm$ ($\sim 8.1 - 10.8 S_A$) in the frontal peatland close to MP6 (**Fig. 2 A and D**), and 7.3 and 11.6 $\mu S/cm$ ($\sim 4.1 - 6.7 S_A$) in the rear peatland close to station MP7 (**Fig. 2 A and E**), thereby demonstrating strongly increased salinities in the freshwater peatland. Changes in salinity can also be observed in other coastal peatlands along the Baltic Sea shoreline. Compared to the occasional increase in salinity in the Hütelmoor induced by storm floods, the Polder Drammendorf was rewetted in November 2019 due to dike removal, which changed the salinity of the partially flooded area from $\sim 2 S_A$ to constant Kubitzer Bodden-like salinities of $\sim 8 S_A$ (unpublished data). Much higher salinities have occurred in the Karrenderfer Meadows. Especially in summer, this coastal peatland experiences strong evaporation with resulting desiccation (**Fig. 4 C**). The remaining puddles along the shoreline increase in salinity due to evaporation, for instance 19 S_A measured at a peatland sampling station approximately 10 m from the shoreline (unpublished data).

With increasing storm-surge events and the subsequent flooding of coastal peatlands, peatland diatoms will be increasingly exposed to higher salinities. However, the euryhaline traits of the peatland strains, with tolerances far exceeding the salinities resulting from storm-surge events, may allow for strong specific growth rates (**Publication II**). This may also be the case for the Baltic Sea species that will be transported into the peatland as the salinity in the peatland increases. Therefore, species such as *Melosira nummuloides*, despite not tolerating 1 S_A (**Publication II**), would be able to grow in peatlands, which after floods have rarely shown salinities less than 5 S_A (**Fig. 7 B**).

3.2.2 Temperature effects on diatom growth

Similarly to the photosynthetic temperature response of the diatom strains studied (**Chapters 3.1.1 and 3.1.2**), the results in **publication II** showed strong specific growth rates for the Baltic Sea and peatland strains over 5 and 30 °C in the 20 % percentile and optimum growth temperatures slightly higher than the average temperatures in the respective habitats. This is in accordance with

observations on other benthic diatoms showing moderate growth between 7 and 27 °C (Baltic Sea, Woelfel et al. 2014) and 4 and 30/40 °C (North Sea, Scholz & Liebezeit 2012a). The seasonal variations in air temperature for the coastal Baltic Sea facing the Hütelmoor and the coastal peatland itself are similar due to their proximity to each other. Nevertheless, the two aquatic habitats have different water-temperature ranges due to the physical properties of each habitat (**Fig. 7 A**). With significantly higher water temperatures in summer and ice cover in the winter, peatlands have a wider range of water temperatures than the Baltic Sea. Despite the differences in temperature of the original habitat of the diatoms, the specific growth rates of the peatland and Baltic Sea strains reported in **publication II**, however, indicate moderate growth within the annual temperature ranges of their own and the adjacent ecosystem.

In comparison of photosynthetic activity to growth, *Nitzschia filiformis* was not able to grow at 5 °C but was still able to perform photosynthesis above the 20 % percentile (**Publication II**). Different temperature tolerances for growth and photosynthesis have also been observed in macroalgae, such as *Fucus vesiculosus* Linnaeus (Graiff et al. 2015). The authors indicated that the time scale of the disruptive stress is critical, as short-term exposure to temperature stress may allow the organism to recover from potential damage under optimal conditions (Eggert et al. 2003). The discrepancy between these results indicates that a brief exposure (40 minutes) to 5 °C will indeed result in a decrease in photosynthetic activity, while a longer exposure (over days) will lead to strongly decreased growth. Incidentally, the results also indicated that, compared to the current measurements (**Fig. 7 A**), an increase in the surface temperature of the southern Baltic Sea by 2 °C predicted for the year 2100 (HELCOM 2013) would not negatively influence the specific growth rates of the Baltic Sea and peatland diatom strains.

3.2.3 Effects of peatland water on diatom growth

As described in the previous chapters, the southern Baltic Sea and the coastal peatlands showed wide fluctuations of light, temperature, and/or salinity. The results detailed in **publications I** and **II** clearly illustrate the wide ecophysiological tolerances of benthic diatoms to fluctuations of these variables. Nevertheless, the results were obtained under laboratory settings, providing controlled conditions of light, temperature, and salinity apart from the tested variable. While it is likely that peatland and Baltic Sea diatom strains will grow and photosynthesize well under natural and climate-change-induced variations of these variables in their respective habitats, so far no studies have investigated how diatoms would respond to blending of the two ecosystems. **Publication III** therefore focused on the specific growth rates, photosynthesis, and respiration of five benthic diatom strains in growth media

simulating the intermixing processes between the Baltic Sea and a coastal peatland, while at the same time simulating abiotic variations in salinity, light, and temperature.

Most likely, the intermixing process will cause a mix of different proportions of Baltic Sea water and peatland water. The results detailed in **publication III** showed an increase in specific growth rates of all five diatom strains with increasing proportions of peatland to Baltic Sea water, ranging from 2 % peatland + 98 % Baltic Sea water to 50 % peatland + 50 % Baltic Sea water.

Intermixing of the freshwater peatland water and the brackish Baltic Sea water led to a change of salinity (**Fig. 7 B**) in the resulting media, ranging between 8 and 15 S_A (**Publication III**). However, compared to the results in **publication II**, with optimum growth rates between 5 and 39 S_A in all five strains, the salinity difference between 8 and 15 S_A was not the determining factor in the large differences in specific growth found for the mixed media in **publication III**. Nevertheless, salinity appears to play an important role for the two Baltic Sea strains *Melosira nummuloides* and *Hyalodiscus* cf. *scoticus*. In contrast to the incubation in peatland water with a salinity of 15 S_A , in which they showed specific growth rates between 0.3 and 0.7 μd^{-1} respectively, the two strains were not able to grow in peatland water at 0.3 S_A . These findings are supported by **publication II**, which reported no growth for *Melosira nummuloides* at a salinity of 1 S_A . Nevertheless, this species was isolated from the Baltic Sea and therefore would have to be transported into a freshwater peatland to experience such a low salinity. However, the salinity was significantly higher after the flooding of the Hütelmoor (Gutekunst et al. 2022, **Fig. 7 B**) and therefore would still allow growth in this peatland after the initial flooding.

Another major difference between the Baltic Sea and the Hütelmoor is the pH (Prelle and Mutinova, unpublished data). However, as pH was fixed between 7.1 and 7.9 (**Publication III**), and since studies of, e.g., the diatom *Skeletonema costatum* (Greville) Cleve showed no effect on diatom growth over a pH range of 6.5 to 8.5 (Taraldsvik & Mykkestad 2000), the effect of pH was not investigated further.

Still, a generally significant difference in specific growth rates was found for the peatland water-based media. All five strains showed maximum species-specific growth rates up to 0.8 μd^{-1} , indicating that components besides the pH and/or salinity stimulated growth. While the exact composition of the peatland water was not determined, two distinct differences between the peatland and Baltic Sea media may have caused higher specific growth rates in the peatland water: the supply of inorganic nutrients and/or organic carbon.

3.2.4 Effects of inorganic nutrients on diatom growth

One of the key differences between the Baltic Sea medium, the peatland medium, and their respective mixed media was the concentration of inorganic nutrients, specifically nitrogen (N), phosphorus (P),

and silica (Si) (**Publication III**). The availability of these nutrients is substantial for diatoms, as they are needed for the characteristic frustule formation, a major component in the DNA and RNA strands, a central component in energy transmission through Adenosine triphosphate (ATP), and synthesis of proteins and enzymes, e.g., RuBisCO, which is responsible for the initial step in CO₂ reduction and fixing of inorganic matter during the photosynthetic metabolism (Brinkmann et al. 2011, Kroth 2015).

The specific growth rates of the five diatom strains, with no significant growth in the Baltic Sea medium, increased significantly with increasing proportions of peatland water, and were highest in 100 % peatland-water medium, reflecting the nutrient availability in the respective media (**Publication III**). With concentrations between 1.2 and 11.2 µmol/L for N (as NO₃⁻, NO₂⁻ and NH₄⁺ combined), 0.1 to 1.2 µmol/L for dissolved and hence bioavailable orthophosphate (**Fig. 7 C and D**), and < 1.0 SiO₂ mg L⁻¹, the content of inorganic nutrients in the Baltic Sea water medium was considerably lower than in the peatland water at < 6.0 SiO₂ mg L⁻¹ with the added 219 µM NaNO₃ as a source of inorganic N and 27 µM NaH₂PO₄·H₂O as a source of inorganic P (**Publication III**). According to the Law of the Minimum (Justus von Liebig), growth in organisms is limited by the scarcest resource (Ebelhar et al. 2008). Therefore, to investigate the possibility that nutrient deficiency was the factor responsible for the lower growth rates with decreasing proportions of peatland water, an additional growth experiment was carried out.

Following the same methodological approach as in **publication III**, using *in-vivo* fluorometry every 24 h for eight d, the specific growth-rate responses of five benthic diatom strains to 18 different nutrient concentrations were determined. Using artificial sea water (15 S_A), omitting the quoted addition of N and P (SAG 2009), 600 µl/l metasilicate (NaSiO₃*5 H₂O; 10 g 100 mL⁻¹) and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer adjusted to a pH of 7.6 as the base medium, ensured precise N and P concentrations and a sufficient Si concentration in each medium, as shown in **Table 1**.

Table 1: Nitrogen and phosphorus concentrations (μM) and N:P ratios of 18 growth media.

Medium	N (as NaNO_3) μM	P (as NaH_2PO_4) μM	N:P ratio
NaCl	-	-	-
M1	10	0.25	40:1
M2	10	0.625	16:1
M3	10	1.25	8:1
M4	10	2.5	4:1
M5	10	10	1:1
M6	20	5	4:1
M7	40	10	4:1
M8	60	15	4:1
M9	70	15	4.6:1
M10	100	12.5	8:1
M11	100	25	4:1
M12	150	18.75	8:1
M13	150	37.5	4:1
M14	200	16.6	12:1
M15	200	25	8:1
M16	300	50	6:1
f/2	880	36	24:1

Similarly to the results in **publication III**, the specific growth rates of all five diatom strains increased significantly with increasing nutrient concentrations, and showed species-specific optima (**Figs. 8 and 9**). Increasing concentrations of N and P $> 100 \mu\text{M}$ N and $12.5 \mu\text{M}$ P (equivalent to M10) generally resulted in only slightly different specific growth rates, indicating nutrient saturation in these media during the experimental phase (**Figs. 8 and 9, Table 1**). Compared to the specific growth rates in the different flood-simulating growth media in **publication III**, the available nutrient concentrations of $219 \mu\text{M}$ N and $27 \mu\text{M}$ P in the peatland-based media provided sufficient nutrients for saturated diatom growth. On the other hand, the specific growth rates in the two mixed media (in proportions of 10 % and 2 % peatland water to Baltic Sea water) indicated an insufficient nutrient supply at the calculated nutrient concentrations of $22 \mu\text{M}$ N and $3 \mu\text{M}$ P, and $5 \mu\text{M}$ N and $< 1 \mu\text{M}$ P, respectively. Therefore, the limiting factor for diatom growth after storm-surge induced mixing of the coastal peatland water and the Baltic Sea water is suggested to be the concentration of inorganic nutrients, in which a lower supply of nutrients results in a lower specific growth rate.

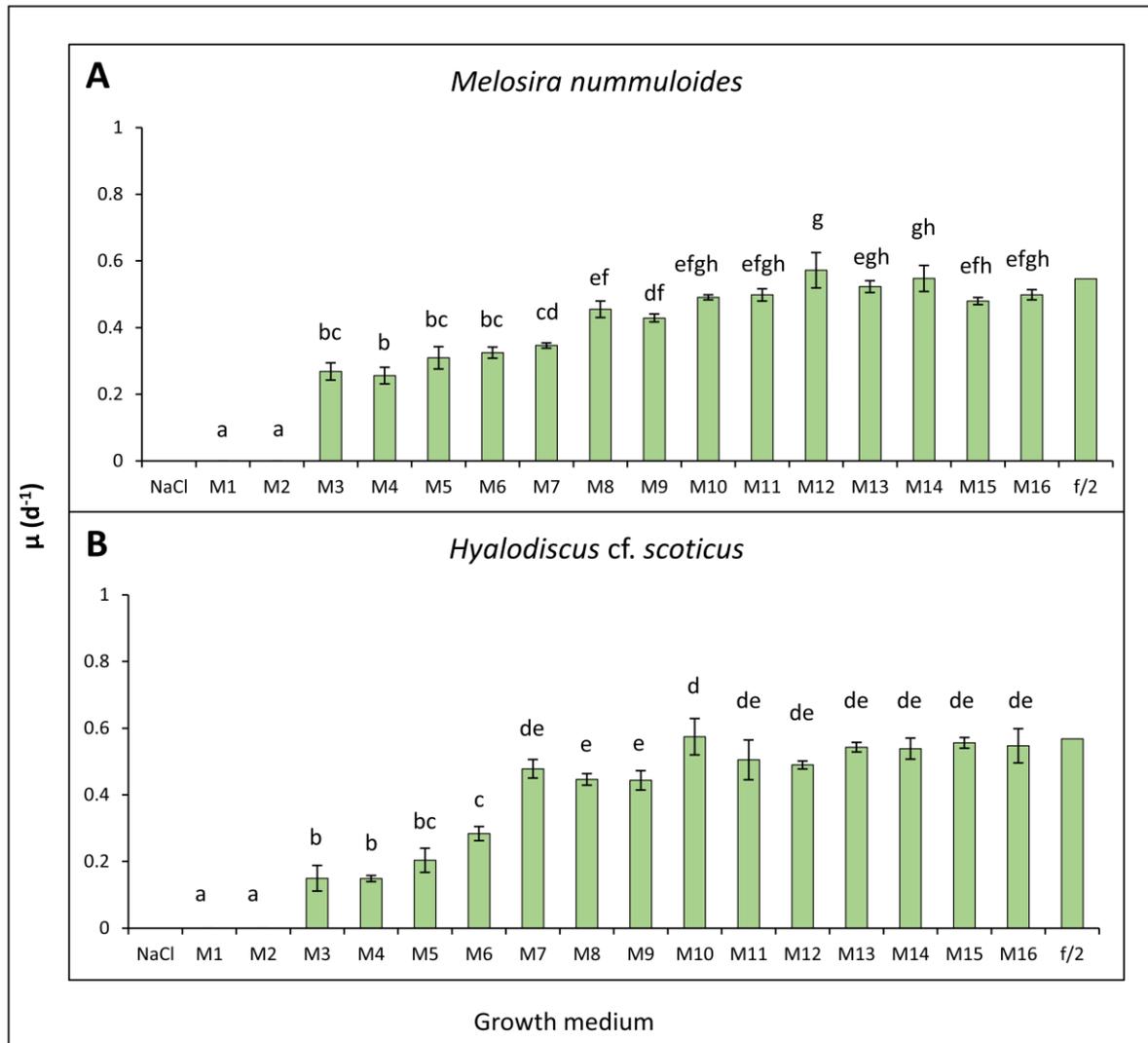


Figure 8 (A – B): Specific growth rates (μ d⁻¹) of two benthic diatom strains isolated from the Baltic Sea, cultured in 18 growth media with different concentrations of nitrogen and phosphorus (Table 1). Data represent means \pm SD ($n = 3$, NaCl and f/2 $n = 1$). Different lowercase letters represent significant levels among all means as calculated by a one-way ANOVA (Tukey's test, $p < 0.05$). **A)** *Melosira nummuloides* and **B)** *Hyalodiscus cf. scoticus*.

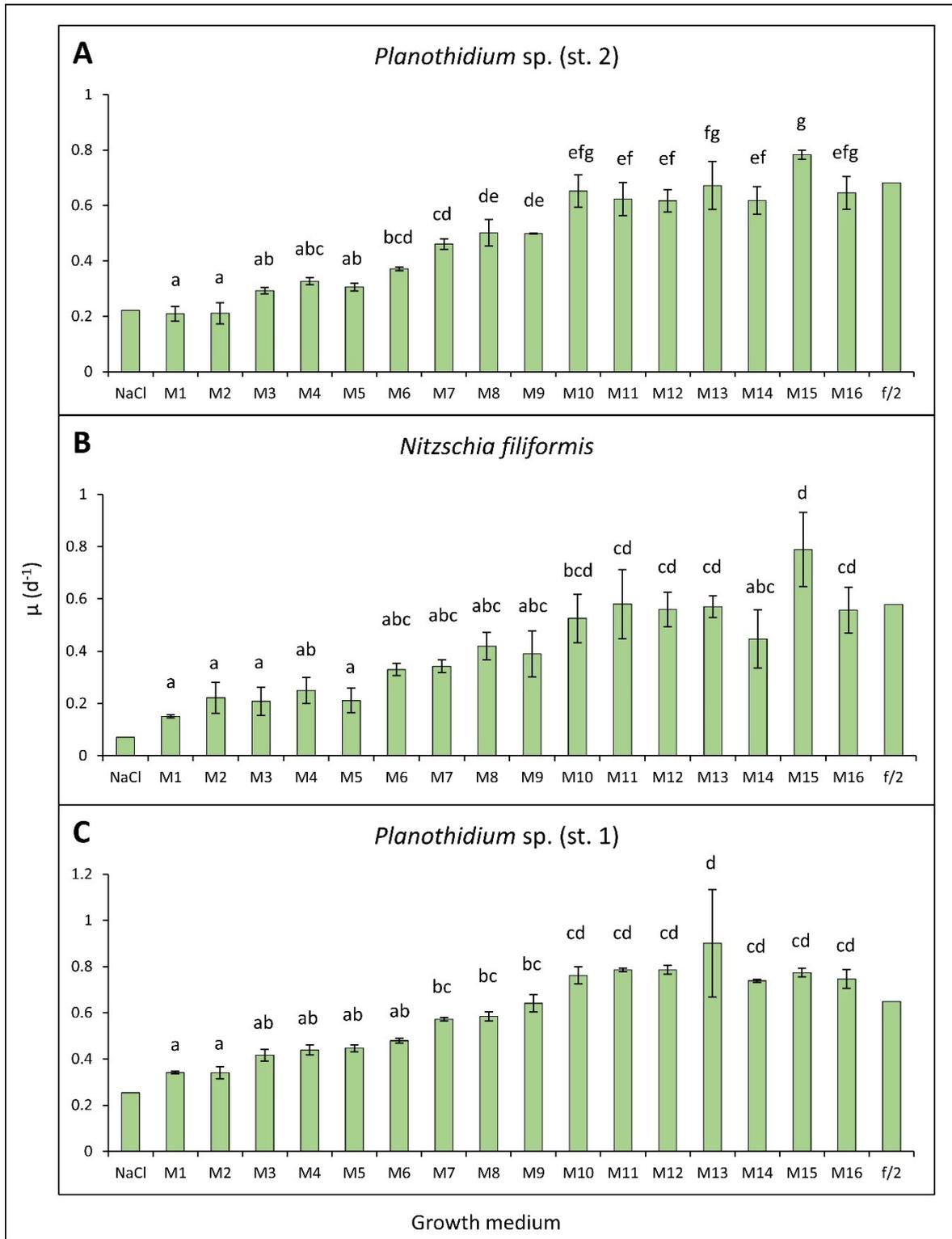


Figure 9 (A – C): Specific growth rates ($\mu \text{ d}^{-1}$) of three benthic diatom strains isolated from three peatlands, cultured in 18 growth media with different concentrations of nitrogen and phosphorus (Table 1). Data represent means \pm SD ($n = 3$; *Nitzschia filiformis* M1 $n = 2$; NaCl and f/2 $n = 1$). Different lowercase letters represent significant levels among all means as calculated by a one-way ANOVA (Tukey's test, $p < 0.05$). **A)** *Planothidium sp. (st. 2)*, **B)** *Nitzschia filiformis*, and **C)** *Planothidium sp. (st. 1)*, notice differently sized axis.

The results described in **publication III** were generated under laboratory conditions and therefore indicate possible effects only to a certain extent. For example, no growth was observed for the two Baltic Sea strains in the Baltic Sea medium, even though it is their originating habitat. Comparing the annual nutrient concentrations, which ranged from 0.1 to 1.2 μM P, and 1.2 to 11.2 μM N (**Fig. 7 C and D**), to the nutrient media with similar concentrations (**Table 1**), the poor specific growth rates of these two strains (**Fig. 8**) may have been caused by a nutrient deficiency. Indeed, while in their natural habitat nutrient supply is often the limiting factor in the surface water (Falkowski & Raven 2013), benthic diatoms can benefit from the nutrient-rich pore water (Admiraal 1984), thereby being supplied with sufficient inorganic nutrients for growth. Leakage of pore water has been reported on the stretch of the Baltic Sea coast facing the Hütelmoor (Jurasinski et al. 2018, Racasa et al. 2021).

At a calculated nutrient concentration of ~ 110 μM N and 14 μM P in the 50 % peatland water + 50 % Baltic Sea water medium used in **publication III** compared to the growth rates shown in **Figs. 8 and 9**, N and P concentrations are presumed to have been sufficient for saturated growth. Still, all five strains showed lower specific growth rates in **Figs. 8 and 9**, compared to the 100 % peatland medium (**Publication III**), indicating that other compounds were responsible for growth differences. In benthic microalgae, the optimum growth medium had an N:P ratio of 17:1 (Hillebrand & Sommer 1999), with other studies showing that growth increased with higher P concentrations (Moorthi et al. 2015). Phosphorus is often the limiting factor for growth, however, the typical ratio of 8:1 or lower found in the peatland (**Fig. 9, Table 1**) suggests that P is not limiting in this habitat. Diatoms are also dependent on a sufficient supply of other nutrients, including Si. However, a deficiency of Si in the growth media described in **publication III** can be ruled out, as specific growth rates were at least similar to if not higher than the growth in nutrient media with sufficient Si supplied (**Publication II**). Therefore, the strong difference in the supply of organic carbon described in **publication I** may have been responsible, potentially initiating heterotrophic rather than photosynthetic growth, as discussed in **Chapter 3.2.6**.

3.2.5 Effects of peatland water on diatom photosynthesis and respiration

Growth of phototrophs is dependent on their photosynthetic metabolism (Marella et al. 2021). While photosynthesis is driven by light as the energy supply, it is also limited by the availability of inorganic nutrients to covert inorganic compounds into organic biomass (Falkowski & Raven 2013). With the differences in specific growth rates between the 50 % mixed medium and the 100 % peatland medium and due to the difference in N and P, it is likely that this would also be reflected in the photosynthesis and respiration rates of the diatom strains. Insufficient supplies of nutrients, especially nitrogen, cause a decline in photosynthetic pigment quantity and PSII activity, as shown for the coastal diatom *Thalassiosira pseudonana* with increased NPQ to counteract potential photo-oxidative damage, which

could be recovered when N was resupplied (Cullen et al. 1992, Liefer et al. 2018). Comparing PI-curves of the diatoms grown in the two media, only *Planothidium* sp. (st. 2) showed significant differences in the photosynthetic rate, with a three-fold increase in the peatland water compared to the mixed medium (**Publication III**). However, the photosynthetic rates were only slightly higher compared to **publication II** when diatoms were grown in f/2 medium (Guillard & Ryther 1962, Guillard 1975) that supplied significantly more N and P. Therefore, it is unlikely that these differences resulted from the differences in nutrient supply.

Beside the supply of inorganic nutrients, as mentioned above, the two media differed widely in availability of organic carbon (**Publication I**). With inadequate nutrients, the photosynthesis rates of *Planothidium* sp. (st. 2) in the 50 % peatland-water mixed medium may therefore have been diminished, as some diatoms are also able to live as facultative heterotrophs, or mixotrophs. For instance, in *Phaeodactylum tricornutum* (Liu et al. 2009, Villanova et al. 2017), CO₂ and organic carbon assimilation proceed simultaneously as competing processes, thereby decreasing the opposing metabolism (Marquez et al. 1993, Villanova & Spetea 2021).

So far, mixotrophy and the effect of organic carbon on the photosynthesis of diatoms have been little studied. Regarding microalgae, Heifetz et al. (2000) found no change in growth rates of the green alga *Chlamydomonas reinhardtii* P. A. Dangeard when supplied with increasing amounts of organic carbon (acetate), although O₂ evolution decreased. Furthermore, Liu et al. (2009) cultured the diatom *Phaeodactylum tricornutum* under phototrophic and mixotrophic conditions, supplying additional acetate, glucose, and glycerol to the growth medium, and found significantly higher growth rates in the mixotrophic media, similar to our findings. This diatom species also showed significantly lower oxygen evolution and increased respiration rates when supplied with organic carbon, which was explained by the loss of photosynthetic pigments (Liu et al. 2009). Significantly lower oxygen evolution under mixotrophic conditions was, however, not confirmed by the findings described in **publication III**. Nevertheless, other studies have observed an increased chlorophyll *a* content in the green alga *Chlamydomonas humicola* Lucksch when grown with the addition of acetate (Lalibertè & de la Noüe 1993), which in turn may increase its photosynthetic activity. Therefore, whether photosynthesis is inhibited or promoted depending on the availability of organic carbon may be species-specific (Liu et al. 2009). However, while peatland habitats are generally high in organic compounds, studies of their exact water composition and organic compounds in this area are lacking, rendering these explanations rather speculative.

3.2.6 Mixo- and heterotrophy in diatoms

While clearly showing specific growth rates related to the nutrient supply (**Chapter 3.2.2**), *Melosira nummuloides*, *Planothidium* sp. (st. 1), and *Planothidium* sp. (st. 2) exhibited significantly higher growth rates in the peatland media, which cannot be ascribed to the supply of N and P (**Publication III**). Due to the significantly higher amounts of dissolved organic carbon in the Hütelmoor compared to the Baltic Sea (**Publication I**), this difference in growth described in **publication III** may be attributed to a mixotrophic and/or heterotrophic metabolism, as also indicated for the Baltic Sea strain *Actinocyclus octonarius* (**Publication I**). Stimulation of mixotrophic growth has been observed in different microalgae, such as the green alga *Apatococcus lobatus* (Chodat) J. B. Petersen, which showed significantly enhanced growth in *Trebouxia* organic medium supplying organic compounds (Gustavs et al. 2016). Although the term “microphytobenthos” suggests primary production, diatoms also show flexibility in trophic growth (Villanova & Spetea 2021), using a mixotrophic or heterotrophic metabolism as the result of e.g., photosynthetic pigment deficiency (obligate), or capability to also perform photosynthesis (facultative) (Villanova et al. 2017). Assimilation of organic carbon under heterotrophic conditions (Villanova & Spetea 2021) has been observed in different diatom species before such as *Cylindrotheca closterium* (Ehrenberg) Reinmann & J. C. Lewis (Saks et al. 1976), *Nitzschia fonticola* (Grunow) Grunow (Kilham et al. 1986) and *Phaeodactylum tricornutum* (Liu et al. 2009).

The preferred sources of organic carbon for diatoms are glucose, glycerol, and acetate (Cerón Garcia et al. 2000, Suman et al. 2012, Zheng et al. 2013); acetate is often found in peatlands (Shannon & White 1996, Ye et al. 2014). Both species of *Planothidium* would therefore be likely to have sufficient organic material for mixo-/heterotrophic growth in their original habitat.

While high concentrations of organic carbon are often found in peatlands, allowing the inhabiting organisms to function as heterotrophs, storm-surge events may cause an influx of organic material into the Baltic Sea, thereby also providing a substrate for mixo-/heterotrophic growth in the Baltic Sea. Additionally, along the Baltic Sea shoreline facing the Hütelmoor, a peat layer extends directly into the sea, discharging submarine groundwater from the Hütelmoor and thereby supplying organic carbon to the coastal water (Racasa et al. 2021). Especially in the Baltic, where wind- and wave-induced sediment turbidity often leads to cell burial, an organic carbon supply via discharge of submarine ground water or direct exchange with coastal peatlands would enable heterotrophic growth in some diatom species, allowing them to grow for an extended period. The ability to use a mixo-/heterotrophic metabolism in response to environmental conditions may also have contributed to the dominance of diatoms in microphytobenthic biofilms.

Comparing the results described in **publications I** and **III**, both applied methods were suitable to indicate mixo-/heterotrophic growth in some of the strains. However, *in-vivo* fluorometry using

chlorophyll *a* as a proxy for biomass in **publication III** is limited in indicating mixo-/heterotrophic growth. As the study by Liu et al. (2009) on *Phaeodactylum tricornutum* showed, the content of chlorophyll *a* in cultures decreased significantly under heterotrophic conditions compared to conditions allowing photosynthesis. In **publication I**, however, a more direct approach used cell counts to determine growth for ten d in complete darkness. As no light was available, the observed growth could be linked directly to a heterotrophic metabolism. Investigation of heterotrophic growth in complete darkness should therefore use the cell-count method, as employed in **publication I**.

3.3 General conclusions

Reflecting on the hypotheses, the following conclusions were reached:

Hypothesis 1:

Baltic Sea and peatland benthic diatoms show photosynthetic and respiratory optima in response to increasing PFD and temperature, reflecting the predominant annual fluctuations in their respective habitats.

This hypothesis can be confirmed. Baltic Sea and peatland benthic diatoms showed different photosynthetic and respiratory optima in response to increasing PFD and temperature, reflecting the prevailing fluctuations in their habitats. However, photosynthetic and respiratory tolerances to PFD and temperature far exceeded the annual ranges of these variables in their respective habitat.

Hypothesis 2:

Baltic Sea and peatland benthic diatoms show growth optima in response to salinity and temperature, reflecting the predominant annual fluctuations in their respective habitats.

This hypothesis can be confirmed. Similarly, to the photosynthetic and respiratory responses, Baltic Sea and peatland benthic diatoms showed growth optima, reflecting the prevailing fluctuations in their habitats. Nevertheless, growth tolerances, especially for salinity, far exceeded the annual ranges of these variables in their respective habitats.

Hypothesis 3:

Increased proportions of peatland water during the intermixing of the two water bodies, Baltic Sea and coastal peatlands, stimulate growth and photosynthesis of the Baltic Sea and peatland benthic diatoms due to the higher concentrations of nitrogen and phosphorus in the peatland water.

This hypothesis can be partially confirmed. Both the Baltic Sea and peatland benthic diatoms showed significantly increased species-specific growth rates in response to increasing proportions of peatland

to Baltic Sea water. However, growth rates at similar or higher inorganic nutrient concentrations were partially lower than specific growth rates in the peatland water-based media. Photosynthesis was not stimulated.

Hypothesis 4:

Peatland water stimulates mixo-/heterotrophic growth in Baltic Sea and peatland benthic diatoms.

This hypothesis can be partially confirmed. Mixo-/heterotrophic growth was indicated in Baltic Sea and peatland benthic diatoms when they were grown in peatland water. This was further reinforced by the significantly increased growth rates of the Baltic Sea strain, *Actinocyclus octonarius*, under heterotrophic conditions in peatland water compared to Baltic Sea water.

Global climate change has inevitable consequences for many aquatic habitats, including the Baltic Sea and coastal peatlands. With ongoing scientific investigations on the underlying hydrodynamic and biogeochemical processes of these environments, environmental developments will become transparent and possible solutions can be found. While the understanding of these processes has significantly increased, it is still in the early stages, as fundamental knowledge of the determining and enlisted components is lacking.

The findings presented in this dissertation therefore contribute to current knowledge, beginning to fill the gap in knowledge of the ecophysiological responses of microphytobenthic diatoms to prevailing abiotic stressors, as major contributors to global primary production and carbon fixation. Showing high photo-physiological plasticity, eurythermal and euryhaline traits far exceeding annual habitat-specific measurements, and the potential to change their metabolisms according to the prevailing conditions, benthic diatoms are not only well acclimated to environmental stressors in their respective habitats but are likely to thrive in climate change-induced abiotic variability. These first findings on the ecophysiology of benthic diatoms in coastal peatlands may help to understand and assess the underlying biological processes involved in renaturation of coastal peatlands. Especially currently, where the carbon footprint is intensively important on a global scale, it is necessary to retrace the impact of biological processes of dominant organisms such as microphytobenthic primary production in order to understand the potential of biological sources to reduce the atmospheric CO₂ concentrations.

3.4 Future perspectives of microphytobenthic diatoms

With the dramatic impact of anthropogenic influences on global ecosystems, understanding the diversity of organisms and the related ecosystem functioning has gained in political and scientific

importance. Especially regarding habitat-specific changes, a comprehensive understanding of underlying processes is essential to counteract negative environmental developments. Due to their abundant cosmopolitan distribution and tolerances/preferences to countless abiotic variables, e.g., pH, salinity, and trophic state (Lobo et al. 2016 and references therein), diatoms have been an effective tool for assessment of the ecological status of environments for years (Stenger-Kovács et al. 2007, Desrosiers et al. 2013), including paleoenvironmental conditions, using diatom fossils (Reid et al. 1995, Serieyssol et al. 2010).

Most of these assessments are based on morphological identification of diatoms, taking advantage of the unique structures of the frustule. However, this assessment process is very time-consuming and requires in-depth taxonomic knowledge. Furthermore, abiotic variables such as salinity strongly affect the morphology of the frustule, resulting in variations in the most important indices, the valve length and species-specific characteristic valve structures, which do not follow a specific pattern (Trobajo et al. 2011). These developments impede the assessment process.

In recent years, morphological determination of diatoms is increasingly accompanied by DNA barcoding of short sections of specific genes, e.g., *rbcL* (Zimmermann et al. 2014), the ITS (internal transcribed spacer) region (Moniz & Kaczmarek 2009), or the 18S V4 region (Zimmermann et al. 2011). The resulting sequences are often deposited in genetic databases such as NCBI, allowing comparison of the DNA/RNA of various diatoms (and other organisms) and species determination apart from morphological identification. Expert knowledge has shown that NGS (next-generation sequencing) technology almost always results in larger numbers of identified species compared to light microscopy (Zimmermann et al. 2015)

Assessment of the ecological status of environments using diatom DNA can strongly benefit by following the long-term goal of environmental DNA (eDNA) barcoding. In brief, this would entail collection of an environmental sample, e.g., sediment, subsequent DNA extraction and amplification using (q)PCR ((quantitative) polymerase chain reaction), and analysis of the resulting sequences using databases. The assembled DNA results would yield a community “fingerprint” as bioindicators, relating taxonomic data to predominant habitat-specific physico-chemical conditions.

In approach, first environmental samples have been collected and partly analyzed in a temporal and spatial time frame. Preliminary unpublished results have revealed the existence of quite different community structures (diversity) in response to flooding/renaturation of coastal peatlands, indicating the potential of using community fingerprints as bioindicators for flooding (Prelle, Mutinova, Dressler & Karsten, unpublished). However, these results were achieved by following a very time-consuming morphological identification approach, which required more than a year to determine the species composition in approximately 100 samples. This strongly supports the potential of eDNA barcoding.

Finally, combining the results of eDNA barcoding and the evolving community fingerprints with the ecophysiological response of specific diatom strains will significantly enhance understanding of the ecosystem functioning, thereby allowing quantitative and qualitative estimates of certain processes, e.g., primary production rates and carbon fixation, which are highly relevant in the age of climate change. In this study, it has become clear that fundamental knowledge of the ecophysiological response of benthic diatoms to habitat-specific environmental stressors is still mostly lacking, leaving room for speculation. Nevertheless, the potential of microphytobenthic diatoms to help understand the ecosystem functioning is tremendous.

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4.1 Publication I

Photosynthesis and Respiration of Baltic Sea Benthic Diatoms to Changing Environmental Conditions and Growth Responses of Selected Species as Affected by an Adjacent Peatland (Hütelmoor)

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Photosynthesis and Respiration of Baltic Sea Benthic Diatoms to Changing Environmental Conditions and Growth Responses of Selected Species as Affected by an Adjacent Peatland (Hütelmoor)

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Eight benthic diatom taxa (*Actinocyclus octonarius*, *Melosira moniliformis*, *Halamphora* sp. 1, *Halamphora* sp. 2, *Navicula perminuta*, *Navicula phyllepta*, *Nitzschia dubiiformis*, *Nitzschia pusilla*) were isolated from sediments sampled in the southern coastal brackish Baltic Sea and established as unialgal cultures. The coastal shallow water sampling area lies close to a fen peat site (Hütelmoor) and both are connected through an underground peat layer, which might facilitate organic matter and nutrient fluxes along the terrestrial-marine gradient. The photosynthetic performance of these diatoms was measured at different photon fluence rates (0–1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, always recorded at 20°C) and different temperatures (5–40°C, always measured at saturating $\sim 270 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), resulting in light saturation points between 32 and 151 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and maximum net primary production rates of 23–144 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl a h}^{-1}$. None of the species showed severe photoinhibition, and hence all displayed a high photo-physiological plasticity. Photosynthetic oxygen evolution and respirational oxygen consumption between 5 and 40°C revealed eurythermal traits for half of the studied taxa as photosynthetic efficiency was at least 20% of the maximum values at the extreme temperatures. The remaining taxa also indicated eurythermal characteristics, however, photosynthetic efficiency of at least 20% was at a narrower temperature range [5 (10) °C to 30 (35) °C]. Species-specific optimum temperatures for photosynthesis (15–30°C) were always lower compared to respiration (25–40°C). *Actinocyclus octonarius* and *Nitzschia dubiiformis* were grown in different defined media, some enriched with Hütelmoor water to test for possible effects of organic components. Hütelmoor water media stimulated growth of both diatom species when kept in a light dark cycle. *Actinocyclus octonarius* particularly grew in darkness in Hütelmoor water media, pointing to heterotrophic capabilities. The benthic diatoms studied are characterized by high photo-physiological plasticity and a broad temperature tolerance to maintain high primary production rates under wide environmental fluctuations.

Organic carbon fluxes from the Hütelmoor into the Baltic Sea may support mixo- and/or heterotrophic growth of microphytobenthic communities. These are essential traits for living in a highly dynamic and variable shallow water environment at the coastal zone of the Baltic Sea.

Keywords: ecophysiology, microphytobenthos, heterotrophy, *Nitzschia dubiiformis*, *Actinocyclus octonarius*

INTRODUCTION

Microphytobenthic assemblages play an important ecological role in marine nearshore ecosystems as they are massively contributing to the marine primary production (Falkowski et al., 1998; Cahoon, 1999). The microphytobenthos is of high diversity as it can be composed of different taxa of euglenids, chlorophytes, cyanobacteria, dinoflagellates, and diatoms (Bacillariophyceae) (Colijn and De Jonge, 1984; Sundbäck and Miles, 2002; Launeau et al., 2018). Many studies, however, have shown that microphytobenthic communities in coastal regions are usually dominated by pennate, often epipelagic motile diatom species, that move freely inside and on the sediment (Hillebrand and Sommer, 1997; Wasmund and Uhlig, 2003; Blommaert et al., 2018). As a result of their carbon fixation capacity, many of these pennate diatoms excrete sticky extracellular polymeric substances (EPS) leading to the formation of phototrophic biofilms on top of soft bottoms, thereby reinforcing the stability of sediments (Goto et al., 2001; De Brouwer et al., 2005; Beninger et al., 2019). In addition, EPS excretion facilitates motility of these protists, for example, through vertical movement into or out of the sediment as protective response to a range of environmental stressors (Cohn and Disparti, 1994; Shniukova and Zolotareva, 2015). Further important ecological functions include those as biological producers for oxygen and filter for other elemental fluxes (e.g., nutrients) at the sediment-water interface (Risgaard-Petersen et al., 1994), and as a major food source for benthic suspension- or deposit-feeders (Cahoon, 1999; Sackett et al., 2016). Benthic diatoms strongly benefit from the usually high nutrient concentrations in the pore water as a source of fuel for photosynthesis and growth (Admiraal, 1984), and they are involved in biochemical cycling of carbon, nitrogen, phosphorous, and silicate in shallow coastal waters (Morel and Price, 2003; Wilhelm et al., 2006).

Shallow coastal zones are quite sensitive to natural changes in abiotic factors due to their distinctive sea-land transition zone which is characterized by exchange processes between the terrestrial and marine compartments. This ecocline typically shows strong diurnal and seasonal fluctuations in meteorological conditions, causing increasing sea-land exchange processes, which might impact organisms living in this transition zone (Jurasinski et al., 2018). In a dynamic environment such as the coastal region of the Baltic Sea, light might be a limiting factor for benthic diatoms due to seasons, meteorological conditions, shading by phytoplankton blooms, turbidity and infauna sedimenturbation. Consequently, photon fluence rates, for example, in the Wismar bight can even diurnally fluctuate between 389 and 2117 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Woelfel et al., 2014). Nevertheless, the light climate in non-tidal coastal areas

like the Baltic Sea seems to be sufficient for benthic diatoms to establish microphytobenthic biofilms (Miller et al., 1996). Depending on the sediment grain size, the thickness of these biofilms can vary as larger grains favor mainly motile diatoms to live between particles. Raphid diatoms vertically move in the sediment with a speed of 10–27 mm h^{-1} in response to limiting or excessive photon fluence rates (Hopkins, 1964; Round, 1971; Perkins et al., 2010).

Besides light, photosynthetic organisms are also dependent on temperature as photosynthesis, respiration and growth are all controlled by this physical factor (Raven and Geider, 1988; Tcherkez et al., 2006). In the shallow coastal area of the Baltic Sea, especially, water surface temperature naturally fluctuates due to seasonality and anthropogenic climate change leads to a warming of up to 0.6 K per decade exceeding the global ocean average by a factor of three (Reusch et al., 2018). Temperature strongly affects EPS production and excretion of temperate benthic diatoms (Wolfstein and Stal, 2002; Aslam et al., 2018), while growth is not influenced in a temperature range between 10 and 30°C (Scholz and Liebezeit, 2012). Net primary production of three benthic diatoms from the southern Baltic Sea increased with decreasing temperatures, and these data were explained by rising contents of photosynthetic enzyme molecules, such as ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) (Mortain-Bertrand et al., 1988; Woelfel et al., 2014). Such a quantitative strategy guarantees to overcome any metabolic constraints imposed by low temperatures, and RuBisCO was identified as the key enzyme involved in such temperature acclimation, while β -carboxylases (e.g., PEP carboxykinase) played a minor role (Mortain-Bertrand et al., 1988). Therefore, the photosynthetic carbon assimilation is controlled by temperature-dependent enzymes (Falkowski and Raven, 1997). The influence of temperature on photosynthesis of benthic diatoms in the Baltic Sea is unstudied (except: Woelfel et al., 2014).

Shallow coastal areas are influenced by terrestrial runoff and may be affected by submarine ground water discharge (Jurasinski et al., 2018). The poor water exchange with the North Sea as well as the influx of agricultural fertilizers and other terrestrial residues has led to eutrophication of the Baltic Sea (Rönnberg and Bonsdorff, 2004). Although the Baltic Sea is characterized as a microtidal ecosystem, strong north-west winds can raise the water level and trigger storm floods at the southern coast. These atmospheric conditions can lead to flooding of coastal peatlands, which contain and store large amounts of carbon (Jurasinski et al., 2018). Peatlands in general store up to 30% of the global carbon by just covering 3% of the entire terrestrial area (Grützmacher, 2009). Coastal peatlands might be connected to the shallow water zone by underground peat layers and submarine groundwater discharge

(Jurasiński et al., 2018), both facilitating the exchange of organic material, minerals and nutrients.

Dissolved organic compounds can be used by heterotrophic organisms to fuel their metabolism. Some microalgae are able to change their metabolic pathways from autotrophy to mixotrophy or even heterotrophy under constant darkness and use of organic compounds as a source of energy (Saks, 1983). Benthic diatom species such as *Nitzschia spec.* increase their production under mixotrophic conditions (Kitano et al., 1997). Heterotrophy in diatoms can either be attributed to a lack of photosynthetic pigments (obligate) or to a temporal separation of photosynthesis and respiration (facultative) like *Cyclotella cryptica* (Villanova et al., 2017). Many diatoms are using a heterotrophic pathway in order to maintain their photosynthetic ability under light-limited conditions, and hence the supply of organic carbon (e.g., glucose, acetate) is essential for long-term survival, for example, when buried in deeper sediment layers after a storm. However, the influence of organic substances from peatlands on benthic diatom growth has not yet been investigated in the southern Baltic Sea.

In the frame of the DFG Research Training Group ‘Baltic Transcoast’¹ a representative coastal site was established as model for transport and transformation processes in the marine and the terrestrial part of the coast, and for evaluating the influence by water and matter inputs from the respective other coastal domain. The study area is the nature reserve “Heiligensee und Hütelmoor,” which is located as coastal fen next to the Baltic Sea near Rostock, Mecklenburg-Western Pomerania, northeastern Germany, on a transition zone between Atlantic maritime and continental climate (Jurasiński et al., 2018). Since the early 2000s the Hütelmoor is part of a restoration project attempting to reach the original condition as a coastal peatland. Therefore, coastal dunes and protection measures separating the Baltic Sea from the peatland will not be maintained. It is anticipated that the natural impact of the Baltic Sea including episodic flooding will re-establish in the near future, most probably resulting in repeated input of brackish waters (on decadal time scales) with possible consequences for hydro-physical and biogeochemical processes (Jurasiński et al., 2018). This will probably increase the exchange and material flows between both systems. In January 2017 a storm flood event already carried brackish water from the Baltic Sea into the Hütelmoor resulting in increasing water levels and salinity (Miegel et al., 2017). Just recently, on 2nd January 2019, another storm flood event caused a break of the main dune and Baltic Sea water flushed into the Hütelmoor. Measurements of salinity have proven saline water intrusion of over 1 km into the peatland (Jurasiński, personal communication), most probably with ecological consequences for the peatland and the exchange processes along the terrestrial-marine gradient. In addition, due to the disappearance of the protective sand dune even modest storm floods exceeding the thresholds of 0.5–1.00 m above sea level will lead to an increasing number of flooding events (Jurasiński et al., 2018). Additionally, there is an underground connection between the Hütelmoor and the shallow water zone as parts of the peat layer are stretching into the Baltic Sea (Jurasiński et al., 2018). Particularly dissolved organic

compounds (DOM) of the peat layer may impact biological activities in the shallow coastal Baltic Sea area (Jurasiński et al., 2018), such as microphytobenthic primary production. DOM was found to be released in higher concentrations from terrestrial peat compared to the outcropping peat layers in the coastal Baltic Sea (Jurasiński et al., 2018). Since ecophysiological traits of clonal benthic diatoms from the Baltic Sea are almost unstudied, this study focused on photosynthesis and respiration of various diatom cultures isolated from the shallow coastal benthos of the southern Baltic Sea in proximity to the Hütelmoor. Furthermore, the impact of Hütelmoor water on growth of unialgal benthic diatoms under light and dark conditions was tested to identify if potential organic carbon sources may support heterotrophy.

MATERIALS AND METHODS

Study Site and Cultures

Benthic diatom strains were isolated from undisturbed sediment cores sampled in nearshore sandy habitats of the Baltic Sea, north east of Rostock in close vicinity to the Hütelmoor (54°13.005'N, 12°9.051'E) at a water depth of 3–6.5 m. Water temperature ranges from 0–3°C during winter up to 20–23°C in summer, and salinity varies between 10 and 15 S_A (H. Lippert, unpublished data). This non-tidal system, however, is very dynamic concerning diatoms occurrences, due to strong mixing of the upper sediment layer at the coast line as well as vertical and horizontal exchange between the land and sea including wind, waves and lateral density gradients (Jurasiński et al., 2018).

From the sediment cores eight diatom species were isolated according to the methodological approach of Stachura-Suchoples et al. (2016). The species included *Actinocyclus octonarius* Ehrenberg (1837), *Melosira moniliformis* (O. F. Müller) Agardh (1824), *Halamphora* sp. 1, *Halamphora* sp. 2, *Navicula perminuta* Grunow (1880), *Navicula phyllepta* Kützing (1844), *Nitzschia dubiiformis* Hustedt (1939) and *Nitzschia pusilla* Grunow (1862), and their morphological features, ecology and identification method (morphology, molecular genetics) are summarized in **Table 1**. Strains were morphologically identified using the taxonomic literature of Snoeijs and Vilbaste (1994), Snoeijs and Potapova (1995), Lange-Bertalot (2000, 2013), and Krammer and Lange-Bertalot (2007, 2010) and were compared to those in the database *Algae Base*² for recent nomenclature. In addition, *N. phyllepta* was molecular-genetically identified according to the protocol of Zimmermann et al. (2011) using the highly variable V4 region of the SSU rRNA, while the *rbcl* gene was used for *A. octonarius*, *M. moniliformis*, *Halamphora* sp. 1, *Halamphora* sp. 2, *N. perminuta*, and *N. pusilla* following Abarca et al. (2014) to at least the genus level. *N. dubiiformis* could not be molecular-taxonomically identified. All sequences were submitted to the National Center for Biotechnology Information (NCBI) under following accession numbers: *rbcl*, *Actinocyclus octonarius*, MN097770, *Halamphora* sp. 1, MN097771, *Navicula perminuta* MN097772, *Nitzschia pusilla*, MN097773, *Melosira moniliformis*, MN097774, *Nitzschia*

¹<https://www.baltic-transcoast.uni-rostock.de>

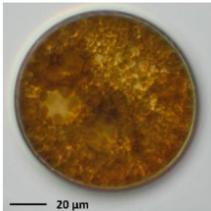
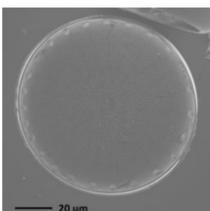
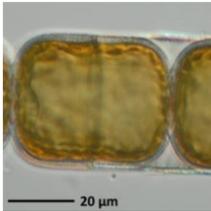
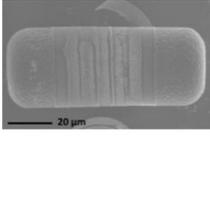
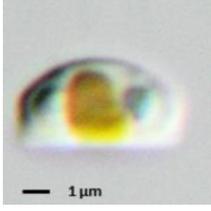
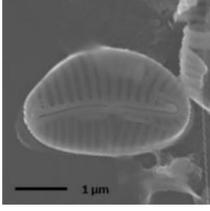
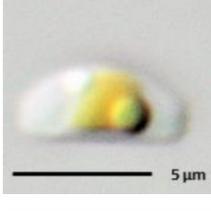
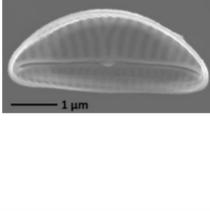
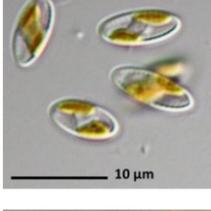
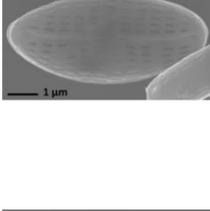
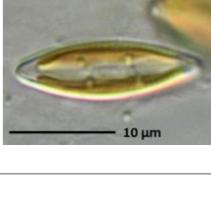
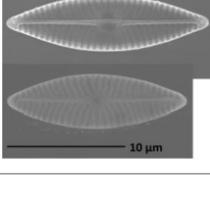
²www.algaebase.org

dubiiformis, MN097775; V4 SSU, *Halamphora* sp. 2, MN097879, *Navicula phyllepta* MH794232.

The diatom isolates were maintained as unialgal, but not axenic stock cultures in Guillard's f/2 medium (Guillard

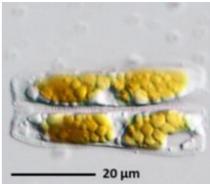
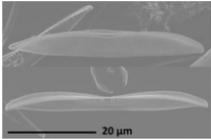
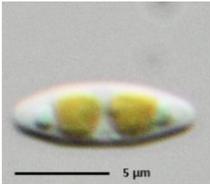
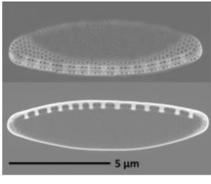
and Ryther, 1962; Guillard, 1975) enriched with metasilicate ($\text{Na}_2\text{SiO}_3 \bullet 5 \text{H}_2\text{O}$; 10 g 100 ml⁻¹) at a final concentration of 0.6 mM. All media were set up with Baltic Sea water (~12 S_A) enriched with additional sea salt (hw Marinemix professional,

TABLE 1 | Morphological and ecological characteristics of eight different diatom strains and identification method (IM) marked with G (genetical) or M (morphological); * marks only genus level.

Isolate	Light microscopy image	SEM image	Size	Ecology	IM
<i>Actinocyclus octonarius</i> , Ehrenberg (1837)			Diameter: 89.04 – 99.77 μm Areolae: 6 – 8 in 10 μm	Pelagic ² , solitary ² , cosmopolitan in marine plankton and coastal zone sediments ⁵	M and G*
<i>Melosira moniliformis</i> , (O. F. Müller) C. Agardh, 1824			Diameter: 35.64 – 46.91 μm Striae: 10 – 12 in 10 μm	Widespread in brackish and marine coastal waters ⁵	M and G
<i>Halamphora</i> sp. 1, Kützing, 1844			Length and width: < 5 μm	Cosmopolitan ³	G*
<i>Halamphora</i> sp. 2, Kützing, 1844			Length and width: < 5 μm	Cosmopolitan ³	M
<i>Navicula perminuta</i> , Grunow in van Heurck, 1880			Length: 5.96 – 8.53 μm Width: 2.08 – 3.56 μm Striae: 14 – 20 in 10 μm Areolae: 33 in 10 μm	Cosmopolitan ^{1,3} , locally abundant in brackish zones of rivers and along the coast ^{3,6}	M and G
<i>Navicula phyllepta</i> , Kützing, 1844			Length: 15.09 – 18.06 μm Width: 4.49 – 6.73 μm Striae: 14 – 20 in 10 μm Areolae: ca. 45 in 10 μm	Epipellic ² , solitary ² , motile ² , brackish water of coastal regions ⁶ , cosmopolitan ³	M and G

(Continued)

TABLE 1 | Continued

Isolate	Light microscopy image	SEM image	Size	Ecology	IM
<i>Nitzschia dubiiformis</i> , Hustedt, 1939			Length: 37.5 – 49.31 µm Width: 3.75 – 6.16 µm Striae: ca. 40 in 10 µm Fibulae: 12–18 in 10 µm	Epipellic and epilithic ² , solitary ² , motile ² , cosmopolitan at marine coasts ⁴	M
<i>Nitzschia pusilla</i> , Grunow, 1862			Length: 9.83 – 10.31 µm Width: 2.43 – 3.67 µm Striae: 43 – 55 in 10 µm Fibulae: 14 – 20 in 10 µm	Epipellic ² , solitary ² , motile ² , cosmopolitan ^{6,4}	M and G

Light microscopic images show the color of the living diatoms and scanning electron microscope (SEM) images show the typical shapes of the frustules. ¹Snoeijs and Vilbaste (1994); ²Snoeijs and Potapova (1995); ³Krammer and Lange-Bertalot (2007); ⁴Krammer and Lange-Bertalot (2010); ⁵Lange-Bertalot (2000); ⁶Lange-Bertalot (2013).

hw Wiegandt Aquaristik, Krefeld, Germany) to achieve always a final salinity of 15 S_A. Cultures were kept at 20°C at a photon fluence density of 30–50 µmol photons m⁻² s⁻¹ under a 16:8 h light:dark cycle (herein referred to as culture conditions). Using this cultivation approach typically resulted in low bacteria numbers ranging of maximum 0.05–0.2% of the diatom biomass.

Light Response Curves (PE-Curves)

Light response (PE) curves were measured with a self-constructed PE-Box using 3 water-tempered DW1 oxygen electrode chambers each placed on a magnetic stirrer (Hansatech Instruments, King's Lynn, United Kingdom) that were kept at a constant temperature of 20°C. Oxygen concentration in the chambers were measured with oxygen dipping probe DP sensors (PreSens Precision Sensing GmbH, Regensburg, Germany) connected to a fiber optic oxygen transmitter via optical fibers (Oxy 4-mini meter, PreSens Precision Sensing GmbH, Regensburg, Germany). Chambers were closed with an air-tight lid. Calibration and measurements were controlled and logged with the PreSens software OXY4v2_30 compatible to the optical transmitter. Before measurements, a two-point calibration (0 and 100% oxygen saturation) was carried out using culture medium.

Three replicates of 3 ml pre-incubated log phase suspension of each diatom culture were filled into each cuvette. To avoid carbon deficiency during measurements, sodium bicarbonate (NaHCO₃, 2 mM final concentration) was added to each cuvette. Diatoms were exposed to ten increasing light levels ranging from 0 to 1249 µmol photons m⁻² s⁻¹ of photosynthetically active radiation (PAR) generated by LEDs (LUXEON Rebel1 LXML-PWN1-0100, neutral-white, Phillips, Amsterdam, Netherlands) implemented into the PE-Box. Light levels were measured with a spherical light sensor (light meter LI-250, LI-COR, Lincoln, United States) placed directly into the cuvette prior to each measurement. Measurements started with a respiration phase of 30 min in the dark followed by a 10 min photosynthesis phase for

each light level. The first and last minute of each phase were not included in the calculations.

P-I curve data were calculated and fitted by the mathematical photosynthesis model of Walsby (1997). The different photosynthetic parameters were estimated from the least-square regression curves fitted to the measured values with the solver function of MS Office excel 2013. From these curves the maximum rate of net primary production (NPP_{max}), respiration (R), light utilization coefficient (α), photoinhibition coefficient (β), light saturation point (I_k) and the light compensation point (I_c) were calculated.

After each PE-curve diatom suspension from each cuvette was filtered onto an individual Whatman GF/6 glass fiber filter (Ø 25 mm) for chlorophyll *a* measurements. Chlorophyll *a* was extracted using 3 ml of 96% ethanol, thoroughly vortexed and incubated for 24 h at 4°C in the dark. Extracts were centrifuged (Heraeus Megafuge 1.0R, Hanau, Germany) for 10 min at 1844 × *g* to decrease turbidity and the supernatant was measured at an extinction of 665 and 750 nm with a spectrophotometer (Shimadzu UV-2401 PC, Kyoto, Japan). Chlorophyll *a* was calculated according a protocol of the Baltic Marine Environment Protection Commission (1988; equation 1).

$$\mu\text{g Chl } a \times l^{-1} = \frac{(E_{665} - E_{750}) \times v \times 10^6}{83 \times V \times d} \quad (1)$$

given *v* as extraction volume (ml), *d* as cell length (cm) and *V* as volume of filtered suspension (ml).

Temperature-Dependent Photosynthesis and Respiration

The effect of rising temperatures on respiratory oxygen consumption and photosynthetic oxygen evolution in the eight diatom isolates was followed between 5 and 40°C in 5°C increments using the oxygen optode system described above and the methodological approach of Karsten et al.

(2010). Beginning with 5°C, all samples were incubated at each experimental temperature for 20 min in the dark before respiration was monitored for an additional 10 min followed by photosynthesis for another 10 min under saturating $267 \pm 7 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR. This photon fluence rate was kept constant for all photosynthesis measurements. After measurement of the photosynthetic oxygen evolution, temperature was increased by 5°C, and after reaching the new temperature in the thermostatic chamber, a new incubation period was started. The oxygen consumption and production per time unit was referenced to the concentration of total Chl *a* per sample as described above. The final photosynthetic and respiratory rates were fitted using the widely applied model of Blanchard et al. (1996), which was originally developed to quantify temperature effects on photosynthesis in microphytobenthic communities. The resulting temperature-response curves were further quantitatively characterized by estimating various descriptive statistics such as minimum, maximum and optimum temperature of photosynthesis and respiration as well as the so-called “performance breath.” The “performance breath” is defined as arbitrary values for the 80 or 20% temperature range which equals “good” or “sufficient” photosynthesis/respiration, respectively, according to the concept of Eggert et al. (2003). These authors introduced the “performance breath” to better distinguish between eurythermal and stenothermal algae.

In order to deeper characterize the photosynthetic and respiratory rates as function of increasing temperature the respective activation energies (E_a) were calculated according to the Boltzmann-Arrhenius function (Padfield et al., 2016).

Diatom Growth in Different Media

The effect of Hütelmoor water on diatom growth (cell counts) was studied using the strains *A. octonarius* and *N. dubiiformis*. Both species were maintained in four different media (sterile filtered): (I) Baltic Sea water (S_A 15), (II) Baltic Sea water enriched with f/2 + Si (as described above; S_A 15), (III) Hütelmoor water salted-up with hw Marinemix professional sea salt (S_A 15), and (IV) salted Hütelmoor water (see III) additionally enriched with f/2 + Si (S_A 15). All media differed in their nutrient and organic component concentrations (Table 2). All experiments were conducted for 10 days under light ($30 - 50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; 16:8 h light:dark) and in parallel under dark conditions. Prior to each experiment both diatom cultures were grown in

fresh culture medium for 10 days to establish log phase biomass. Afterward, Erlenmeyer flasks were filled with the respective medium and inoculated with an always equal amount of cells per ml of the respective stock culture. Algal growth was determined in three replicate 3 ml samples preserved in Lugol's iodine solution (Thronsdon and Sournia, 1978) that were taken at the beginning as well as at the end of the experiment. Samples were counted in sedimentation chambers (area: 31.42 mm^2) using an inverted microscope (Olympus IX70, Olympus, Hamburg, Germany) following the guidelines of Utermöhl (1958), high-density samples were counted up to 400 cells per sample. Cell number was always calculated per milliliter volume.

Each medium was filtered through a $0.45 \mu\text{m}$ cellulose acetate membrane filter (Sartorius, Goettingen, Germany) for analysis of phosphate (PO_4^{3-}), nitrate (NO_3^-) and ammonium (NH_4^+). PO_4^{3-} and NO_3^- were measured using a continuous-flow analyzer (Alliance Instruments, Salzburg, Austria) following the manufacturer's protocol for both compounds. NH_4^+ was measured manually at 630 nm using a spectrophotometer after reaction of samples with a mixed phenol solution (3.5 g phenol and 0.04 g nitroprusside sodium dissolved in 100 ml demineralized water) and Trion solution (0.25 g Trion filled up to 100 ml citrate buffer solution) (DIN 38406 E5-1). The spectral absorption coefficient at 254 nm (SAC_{254}) was determined as a proxy for dissolved organic compounds in the applied growth media using a spectrophotometer (Shimadzu UV-2401 PC, Kyoto, Japan). Furthermore, a visual aquarium quick assay for silicate was performed (JBL, Neuhofen, Germany) according to the manufacturer's protocol. The amount of silicate was categorized in nine color shades from <0.1 to $>6.0 \text{ SiO}_2 \text{ mg l}^{-1}$.

Statistical Analysis

All calculations were undertaken using Microsoft Office Excel (2013). All values shown represent the mean value of three replicates. Significance levels were calculated using one-way ANOVA followed by a *post hoc* Tukey's honest significant difference test (critical *p*-value < 0.05). Prior to the analysis, data were tested for normality using the Shapiro-Wilk's test and for homogeneity of variances using Levene's test. Statistical analysis of the data was performed using SPSS Statistics 22 (IBM, Armonk, NY, United States).

RESULTS

PE-Curves

The benthic diatoms studied showed species-specific respiratory oxygen consumption rates in the dark as well as photosynthetic oxygen evolution rates with increasing photon fluence rates up to $\sim 1250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figures 1A–H). A potential contribution by diatom-associated bacteria to the oxygen signals can be neglected because of their always low cell numbers (less than 0.05–0.2% of diatom biomass) during exponential growth phase and during experimentation. Using the photosynthetic model of Walsby (1997), characteristic *PE*-curve parameters were calculated (Table 3). The dark respiration rates in most species were in a similar range (-17.1 to $-29.2 \mu\text{mol O}_2 \text{ mg}^{-1}$

TABLE 2 | Analysis of SAC_{254} , salinity, phosphate (PO_4^{3-}), ammonium (NH_4^+), and nitrate (NO_3^-) in $\mu\text{mol/L}$ for respective medium Baltic Sea water (Baltic), f/2 enriched Baltic Sea water (Baltic and f/2), salted Hütelmoor water (salted), and f/2 enriched Hütelmoor water (salted and f/2).

Medium	PO_4^{3-} $\mu\text{mol/L}$	NH_4^+ $\mu\text{mol/L}$	NO_3^- $\mu\text{mol/L}$	N:P	SAC_{254} (1/m)	Salinity (S_A)
Baltic Sea	1.5	0.65	0.64	0.87	13	15.0
Baltic Sea + f/2	23.7	0.73	943	39.82	–	15.0
Hütelmoor	34.8	12.53	139	4.35	283	15.0
Hütelmoor + f/2	62.8	9.39	929	14.94	–	15.0

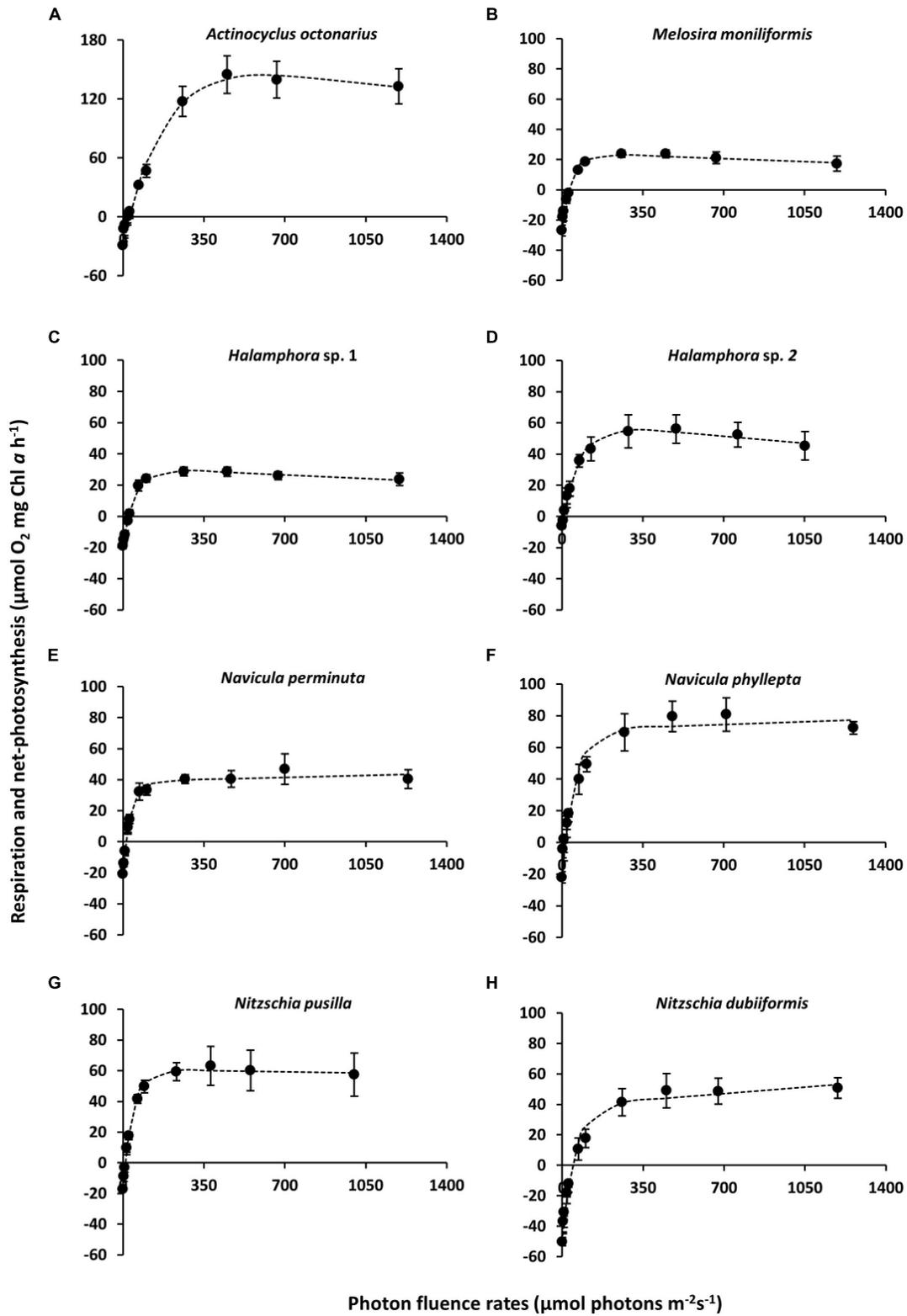


FIGURE 1 | Continued

FIGURE 1 | (A–H) Photosynthesis and respiration rates ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$) in relation to increasing photon flux density ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) of eight diatom cultures – kept at 20°C in f/2 Baltic Sea water medium, 15S_A – measured by oxygen evolution with optodes. Data represent mean values \pm SD ($n = 3$). **(A)** *Actinocyclus octonarius*, notice differently sized axis; **(B)** *Melosira moniliformis*; **(C)** *Halamphora* sp. 1; **(D)** *Halamphora* sp. 2; **(E)** *Navicula perminuta*; **(F)** *Navicula phyllepta*; **(G)** *Nitzschia dubiiformis*; **(H)** *Nitzschia pusilla*.

TABLE 3 | Parameter of respective PE-curves (**Figures 1A–H**) of eight diatom cultures ($n = 3$) kept at 20°C in a f/2 Baltic Sea water medium, 15S_A.

Isolates	NPP _{max} ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$)	Respiration ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$)	α ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$) ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) ⁻¹	β ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$) ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) ⁻¹	I _k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	I _c ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	NPP _{max} : Respiration
<i>Actinocyclus octonarius</i>	144.07 \pm 19.47 a	-29.21 \pm 1.30	1.15 \pm 0.14	-0.03 \pm 0.01	151.34 \pm 9.51	28.5 \pm 2.20	4.93 \pm 0.54
<i>Melosira moniliformis</i>	23.12 \pm 1.62 b	-26.96 \pm 3.55	1.26 \pm 0.18	-0.01 \pm 0.00	39.78 \pm 2.31	30.57 \pm 3.12	0.86 \pm 0.08
<i>Halamphora</i> sp. 1	29.31 \pm 2.68 bcd	-19.01 \pm 2.59	1.01 \pm 0.24	-0.01 \pm 0.00	48.05 \pm 5.52	24 \pm 2.29	1.54 \pm 0.07
<i>Halamphora</i> sp. 2	55.46 \pm 10.62 cde	-6.07 \pm 6.18	0.88 \pm 0.15	-0.01 \pm 0.01	69.75 \pm 7.96	7.32 \pm 3.35	9.14 \pm 0.22
<i>Navicula perminuta</i>	38.73 \pm 2.86 bcd	-20.84 \pm 4.81	1.88 \pm 0.58	0.01 \pm 0.01	31.61 \pm 2.22	13.49 \pm 1.26	1.86 \pm 0.08
<i>Navicula phyllepta</i>	70.81 \pm 7.89 e	-22.15 \pm 14.67	1.64 \pm 0.76	0.01 \pm 0.00	56.66 \pm 12.62	15.36 \pm 3.61	3.20 \pm 0.56
<i>Nitzschia dubiiformis</i>	38.60 \pm 12.08 b	-50.18 \pm 3.33	1.6 \pm 0.63	0.01 \pm 0.01	55.55 \pm 12.76	45.50 \pm 6.24	0.77 \pm 0.19
<i>Nitzschia pusilla</i>	60.12 \pm 12.03 d	-17.11 \pm 2.85	1.84 \pm 0.15	-0.02 \pm 0.00	41.93 \pm 9.32	10.50 \pm 0.45	3.51 \pm 0.19

NPP_{max} represents the maximal oxygen production rate, alpha the initial slope of production in the light limited range, beta the terminal slope of production in extensive light range (photoinhibition), I_k the light saturation point, I_c the light compensation point. Data represent a mean value \pm SD ($n = 3$). Different lowercase letters represent significance levels among all means as calculated by a one-way ANOVA (Tukey's test, $p < 0.05$). NPP_{max}: $F = 29.576$, $df = 4$, $p < 0.001$.

Chl *a* h⁻¹), except in *Halamphora* sp. 2 and *N. dubiiformis* that exhibited much lower or higher rates with -6.1 and -50.2 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$, respectively (**Table 3**). A similar broad range was measured for the maximum photosynthetic rates (NPP_{max} 23.1 to 144.1 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$) in the 8 diatom species, while the light utilization coefficient (α), which reflects photosynthetic efficiency, was with 0.88 to 1.88 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)⁻¹ rather similar (**Table 3**). Light compensation points (I_c) were between 7.3 and 45.5 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and the light saturation points (I_k) ranged from 31.6 to 69.8 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in 7 out of 8 species (**Table 3**). In contrast, *A. octonarius* exhibited with 151.3 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ a 2–5 times higher I_k value. The Walsby model fit indicated that 5 out of 8 species showed slight photoinhibition under the highest photon fluence rate tested [β : -0.01 to -0.03 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)⁻¹], while the remaining 3 species (*N. perminuta*, *N. phyllepta*, *N. dubiiformis*) were not photoinhibited at all (**Table 3**). NPP_{max} to respiration ratios ranged from 0.8 to 9.1 (**Table 3**).

Temperature-Dependent Photosynthesis and Respiration

The effect of increasing temperature from 5 to 40°C on net photosynthetic oxygen evolution and respiratory oxygen consumption revealed strong differences between both physiological processes and among the benthic diatoms studied (**Figures 2A–H**). Rising temperature stimulated photosynthesis and respiration up to a species-specific maximum followed by a decline under the highest temperature conditions. While optimum photosynthesis was measured between 10 and 30°C among all species studied, highest respiration occurred between 25 and 40°C (**Figures 2A–H**).

Photosynthetic oxygen evolution of *N. phyllepta* (**Figure 2F**) and *N. dubiiformis* (**Figure 2G**) significantly increased from 5 to 20°C/25°C, the latter temperatures reflecting the species-specific optimum. Further increase in temperature to 30°C was accompanied by a 30–40% decline in photosynthesis, while incubation at 35 and 40°C even led to complete inhibition as reflected in negative values for oxygen evolution. Respiratory oxygen consumption in both species increased between 5 and 30°C (**Figures 2F,G**). Further rise in temperature (35 and 40°C) led to a significant decrease in respiratory activity. The maximum photosynthetic oxygen evolution of *N. dubiiformis* was (137 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$) about twice of that in *N. phyllepta* (65 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$). In addition, the maximum respiratory oxygen consumption of *N. dubiiformis* (-58 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$) was also twofold higher compared to *N. phyllepta* (-30 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$) (**Figures 2F,G**).

The photosynthetic oxygen evolution in *Halamphora* sp. 2 (**Figure 2D**) and *N. pusilla* (**Figure 2H**) also increased with rising temperatures. While *Halamphora* sp. 2 showed a broad optimum between 15 and 25°C, the second species exhibited maximum photosynthesis at 30°C. Higher temperatures of 35 and 40°C led in both strains to a decline of photosynthesis, but not to complete inhibition (**Figures 2D,H**). Maximum photosynthesis of *Halamphora* sp. 2 was almost 1.7-fold higher (129 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$) compared to *N. pusilla* (77 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$). Respiratory oxygen consumption in both taxa displayed similar trends as respiration rates increased with rising temperatures up to -40 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ at 25 and 30°C, respectively, and remained on this level with further rising temperatures up to 40°C (**Figures 2D,H**).

In contrast to the previously described species, *Halamphora* sp. 1 and *N. perminuta* (**Figures 2C,E**) exhibited generally much lower values of maximum photosynthetic oxygen evolution (14–39 $\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$). Both strains showed increasing

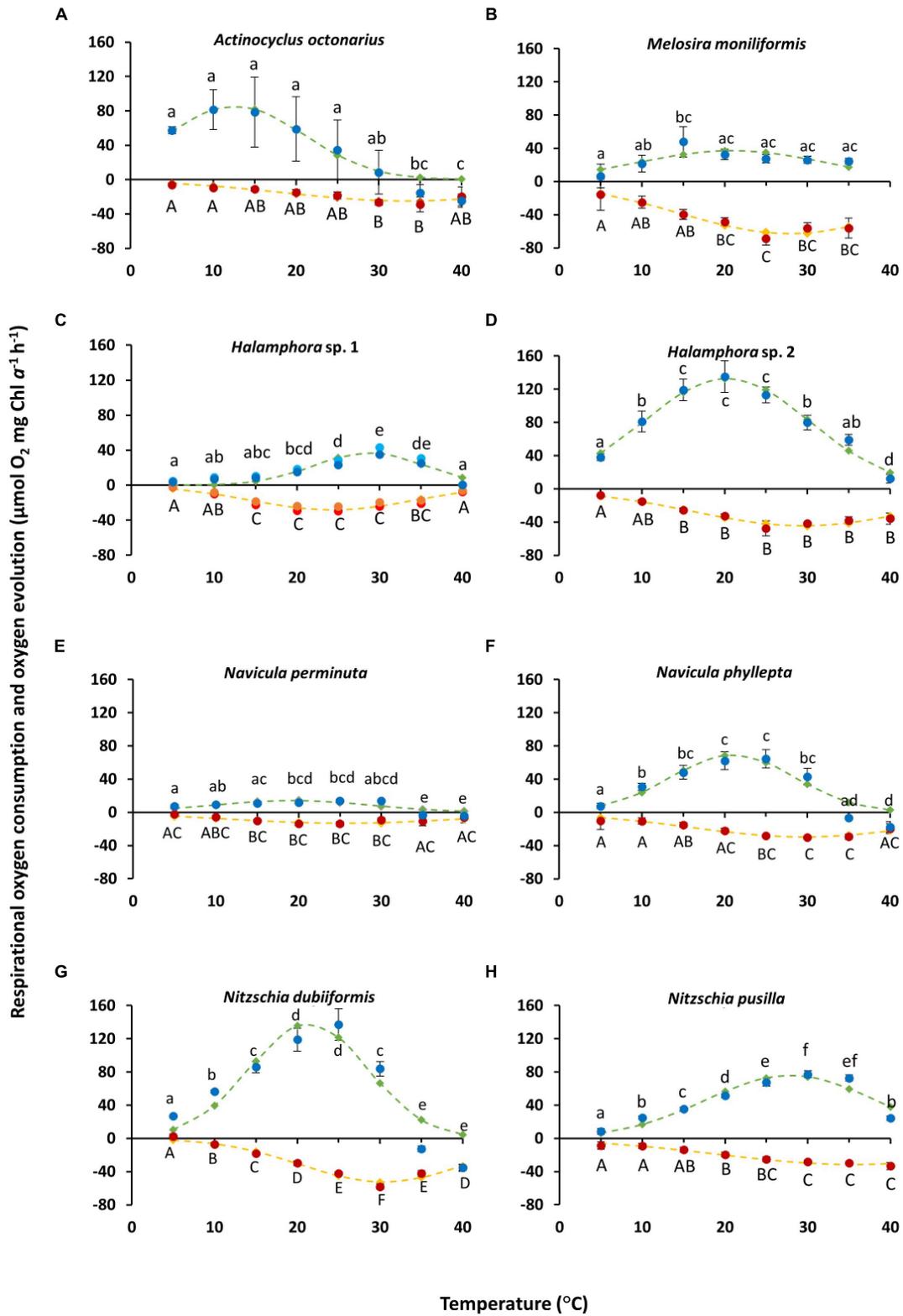


FIGURE 2 | Continued

FIGURE 2 | (A–H) Photosynthetic (blue) oxygen evolution at $266 \pm 6.7 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ and respiratory (red) oxygen consumption in darkness of eight different diatom cultures as a function of increasing temperature. The measured data were fitted by the model of Blanchard et al. (1996) (photosynthesis: green dashed line; respiration: yellow dashed line). All cultures were kept in *f/2* Baltic Sea water medium, 15_{SA} . Data represent mean values \pm SD ($n = 3$). Different lowercase (photosynthesis) and capital letters (respiration) indicate significantly means ($p < 0.05$; one-way ANOVA with *post hoc* Tukey's test). **(A)** *Actinocyclus octonarius*; **(B)** *Melosira moniliformis*; **(C)** *Halamphora* sp. 1, notice values consist of $n = 2$ (represented each by different colors), **(D)** *Halamphora* sp. 2; **(E)** *Navicula perminuta*; **(F)** *Navicula phyllepta*; **(G)** *Nitzschia dubiiformis*; **(H)** *Nitzschia pusilla*.

photosynthesis rates with rising temperatures and optimum rates at 30°C , followed by a strong decline at 35°C (Figures 2C,E). At 40°C photosynthesis was completely inhibited. Respiratory oxygen consumption rose with increasing temperatures up to 30°C , followed by a slight decrease with further rising temperature (Figures 2C,E).

Photosynthetic oxygen evolution in *M. moniliformis* increased between 5 and 15°C up to $48 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$, with the optimum at 15°C (Figure 2B). Further rise in temperature was accompanied by a 45% decline in photosynthesis, which, however, remained constant between 20 to 40°C at 24 – $27 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ (Figure 2B). Respiratory oxygen consumption linearly increased from 5 to 25°C up to $-60 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$, followed by a slight decrease at 30 and 35°C (Figure 2B). Due to technical malfunctions, no measurements were made at 40°C for *M. moniliformis*.

Actinocyclus octonarius showed highest photosynthetic oxygen evolution at lower temperatures (5 to 25°C), followed by a strong to complete inhibition of photosynthesis between 30 and 40°C (Figure 2A). The respiratory oxygen consumption slowly increased from 5 to 30°C , with an optimum at 30 – 35°C , followed by a slight decline at 40°C (Figure 2A).

In addition, the optimum temperatures for photosynthesis and respiration were also calculated using the widely used model of Blanchard et al. (1996) (Figures 2A–H, green and yellow dashed lines), which provided more precise values. For five species (*M. moniliformis*, *Halamphora* sp. 2, *N. perminuta*, *N. phyllepta*, *N. dubiiformis*) optimum temperature for photosynthesis was calculated between 19.2 and 21.4°C which reflects the upper temperature range of their natural habitat (Table 4). While for *N. pusilla* and *Halamphora* sp. 1 higher temperature optima for photosynthesis between 27.9 and at 28.8°C were calculated, *A. octonarius* exhibited lower temperatures in the model (12.6°C) (Table 4). At the upper 80% percentile, modeled data of the optimum temperature for photosynthesis confirmed those of the measurements. In contrast, at the upper 20% percentile a broader temperature range was calculated by the model compared to the measurements. Calculation of the optimum temperature for respiration resulted in evenly distributed rates among the eight diatom strains, ranging from 24.3°C in *Halamphora* sp. 1 to 35.7°C in *N. pusilla* (Table 4). The model data confirmed the higher temperature requirement for respiration compared to photosynthesis, as well as a broader temperature tolerance as reflected in the upper 80% percentile and the upper 20% percentile (Table 4).

From the photosynthesis and respiration data in Figures 2A–H the species-specific temperature tolerance width for both physiological processes was calculated as percentiles of $< 0\%$, 0 – 19% , 20 – 80% , and $> 80\%$ of the optimum values (Figures 3A,B).

All benthic diatoms showed a wide range of temperature tolerance for photosynthesis and respiration with almost all values surpassing the 20% percentile, and hence the eight species can be characterized as eurythermal organisms.

All benthic diatom species photosynthesized at 5°C , but only 3 out of 8 taxa were also capable to do so at 40°C (both *Halamphora* strains, *N. pusilla*). Half of the studied species exhibited photosynthesis up to 30°C , while at 35°C and 40°C complete inhibition was determined (Figure 3A). *Melosira moniliformis* showed photosynthesis up to 35°C . *Actinocyclus octonarius* and *M. moniliformis* had their photosynthetic optima at lower temperatures between 10 and 15°C , while all other species between 15 and 30°C . Both *Halamphora* species markedly differed in their photosynthesis-temperature patterns. While *Halamphora* sp. 1 showed a distinct optimum at 30°C , that of *Halamphora* sp. 2 shifted toward lower temperatures and was rather broad between 15 and 25°C (Figure 3A).

Except *N. dubiiformis* all other benthic diatom species respired at 5°C , and 6 out of 8 taxa were also capable to do so at 40°C . *Melosira moniliformis* and *Halamphora* sp. 1 showed respiration only up to 35°C (Figure 3B). Compared to photosynthesis, the upper percentile of $> 80\%$ for respiration was always at higher temperatures, ranging from 15 to 40°C . The respiratory activity optimum of *Halamphora* sp. 2, *N. pusilla*, *N. phyllepta*, and *M. moniliformis* was between 25 and 35°C , partly overlapping with their photosynthesis optima. *Halamphora* sp. 1 revealed widest optimum respiration activity ($> 80\%$ at 15 – 30°C) among the tested isolates. Overall, also concerning respiration all benthic diatom species can be characterized as eurythermal organisms. Thermal response calculations indicated species-specific activation energy (E_a) for photosynthesis as well as for respiration among the tested benthic diatom species. *Navicula perminuta* showed lowest E_a for photosynthesis (0.22 eV) and *M. moniliformis* exhibited the highest value (1.37 eV). Both *Halamphora* and both *Nitzschia* species had almost identical E_a values ranging from 0.58 to 0.62 eV (Table 4). Activation energies for respiration were between 0.38 and 1.92 eV among all benthic diatom taxa. In contrast to photosynthesis both *Halamphora* and both *Nitzschia* species showed stronger differences in E_a values for respiration (*Halamphora*: 0.63 and 0.98 eV ; *Nitzschia*: 0.39 and 1.92 eV) (Table 4).

Diatom Growth in Different Media

In the growth experiments with *A. octonarius* the initial cell density for each treatment was with ca. 40 cells ml^{-1} highly similar, and after 10 days incubation an increase of cell number was observed in all media (Figure 4A). Cells grew under light and dark conditions, depending on the media used. Light treatment and incubation in media enriched with *f/2* supplement resulted

TABLE 4 | Calculation of temperature effects on photosynthetic oxygen evolution (A) and respirational oxygen consumption (B) of eight tested benthic Baltic Sea diatom strains using the Blanchard et al. (1996) fit; additionally, the respective activation energy (E_a) is given.

	dO ₂ (μmol O ₂ mg ⁻¹ Chl a h ⁻¹)			Temperature (°C)			E _a (eV)
	100%	>80%	>20%	100%	>80%	>20%	
(A) Photosynthesis							
<i>Actinocyclus octonarius</i>	85	> 68.06	> 17.01	12.6	7 – 18.2	(–2.5) – 27.6	0.47
<i>Melosira moniliformis</i>	37.17	> 29.74	> 7.43	20.9	13.2 – 28.6	(0.2) – (41.5)	1.37
<i>Halamphora</i> sp. 1	36.92	> 29.54	> 7.38	28.8	24.3 – 33.2	16.8 – (40.7)	0.62
<i>Halamphora</i> sp. 2	132.85	> 106	> 26.57	20.3	13.5 – 27	(2.0) – 38.4	0.59
<i>Navicula perminuta</i>	14.32	> 11.46	> 2.86	19.2	12.9 – 25.6	(2.2) – 36.2	0.22
<i>Navicula phyllepta</i>	68.97	> 55.18	> 13.79	21.0	15.9 – 26.0	7.4 – 34.5	0.72
<i>Nitzschia dubiiformis</i>	138.11	> 110.49	> 27.62	21.4	16.6 – 26.1	8.5 – 34.2	0.58
<i>Nitzschia pusilla</i>	75.67	> 60.54	> 15.13	27.9	21.0 – 34.8	9.4 – (46.3)	0.60
(B) Respiration							
<i>Actinocyclus octonarius</i>	–25.07	< –20.06	< –5.01	33.4	23.5 – (43.4)	6.6 – (60.1)	0.38
<i>Melosira moniliformis</i>	–62.85	< –50.28	< –12.57	27.9	19.0 – 36.8	(3.9) – (51.8)	0.52
<i>Halamphora</i> sp. 1	–28.04	< –22.43	< –5.61	24.3	17.7 – 31.0	6.4 – (42.1)	0.98
<i>Halamphora</i> sp. 2	–44.64	< –35.71	< –8.93	29.5	20.5 – 38.5	5.3 – (53.6)	0.63
<i>Navicula perminuta</i>	–13.11	< –10.49	< –2.62	25.5	16.0 – 35.0	(–0.1) – (50.9)	0.67
<i>Navicula phyllepta</i>	–29.41	< –23.53	< –5.88	29.6	20.4 – 38.8	(4.8) – (54.2)	0.38
<i>Nitzschia dubiiformis</i>	–52.59	< –42.07	< –10.52	30.4	23.8 – 37.0	12.06 – (48.2)	1.92
<i>Nitzschia pusilla</i>	–31.35	< –25.08	< –6.27	35.7	24.6 – (46.8)	5.8 – (65.4)	0.39

Data represent mean values ($n = 3$) of the maximum photosynthesis and respiration (100%) as well as the upper 80 and 20% percentile. Data in brackets represent temperature calculation exceeding measured temperature.

in highest increase in cells densities (Baltic Sea water 200 cells ml⁻¹, Hütelmoor water 250 cells ml⁻¹). Baltic Sea water without $f/2$ led to 67 cells ml⁻¹ and Hütelmoor water without $f/2$ to 100 cells ml⁻¹ under both light and dark conditions. In the dark, the addition of $f/2$ did not significantly increase cell density in the Baltic Sea water nor in the Hütelmoor medium compared to the pure medium (Figure 4A). Nevertheless, both Hütelmoor water media resulted in a two-fold increase of cell number in darkness compared to pure Baltic Sea water in the dark and in the light.

The initial cell numbers of *N. dubiiformis* differed significantly among treatments with 1.400 to 4.000 cells ml⁻¹ (One-way ANOVA with *post hoc* Tukey's test, $p < 0.05$). Cells grew only in the light, with significant differences depending on the media used (One-way ANOVA with *post hoc* Tukey's test, $p < 0.05$) (Figure 4B). This species did not grow in pure Baltic Sea water. The highest increase in cell number was observed in the Hütelmoor water enriched with $f/2$ (400.000 cells ml⁻¹), while Baltic Sea water enriched with $f/2$ resulted in about 300.000 cells ml⁻¹. Hütelmoor water without $f/2$ stimulated cell growth up to 100.000 cells ml⁻¹ (Figure 4B).

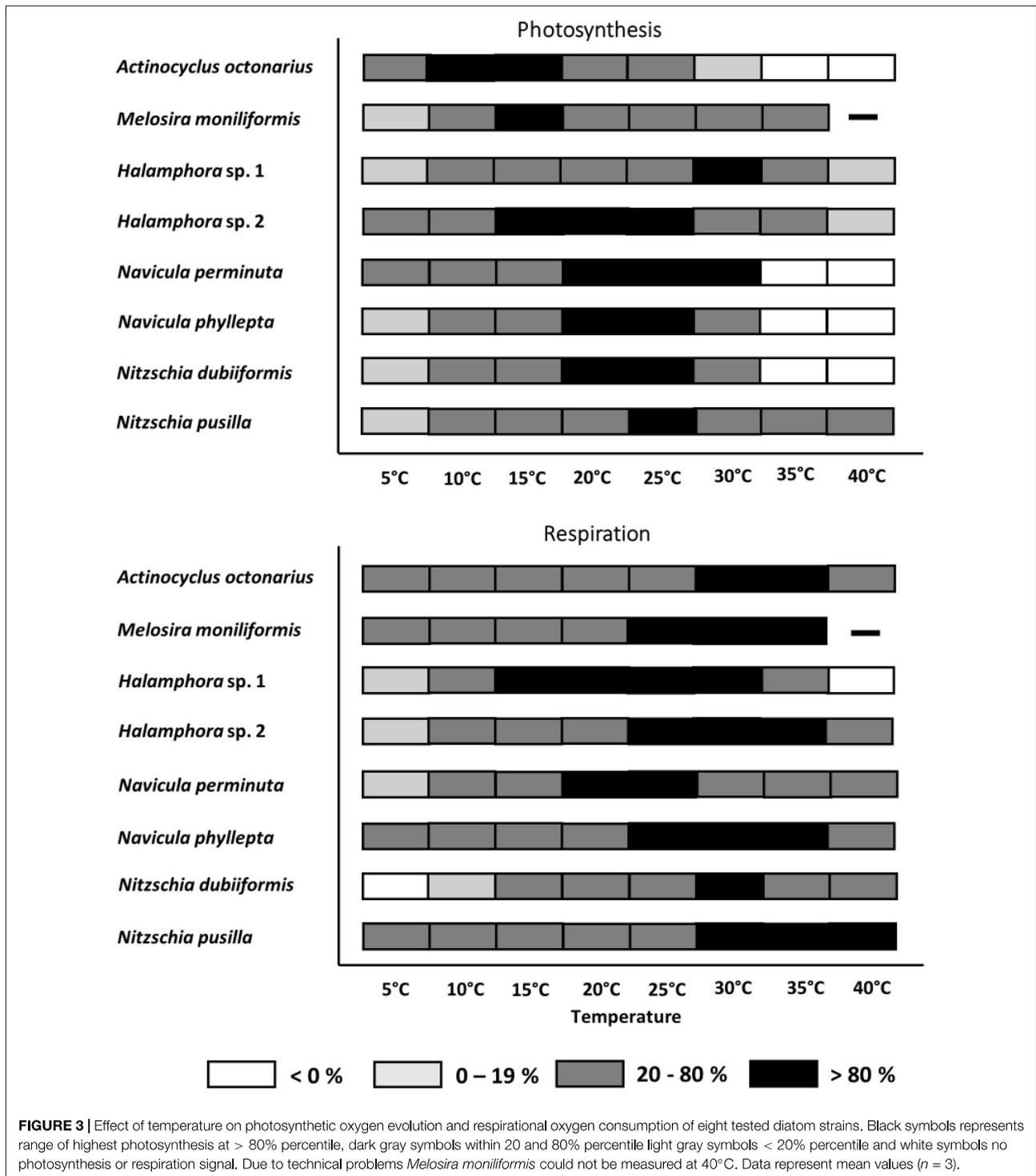
DISCUSSION

The eight benthic diatom species from the shallow coastal water zone of the southern Baltic Sea showed a broad photosynthetic performance at different photon fluence rates from 0 to 1250 μmol photons m⁻² s⁻¹ as well as at different temperatures from 5 to 40°C. These data point to rather high photo-physiological plasticity since

in five out of eight species only minor photoinhibition was observed, while the remaining strains were not photoinhibited at all. In addition, the temperature tolerance was generally broad characterizing the benthic diatoms studied as eurythermal organisms. Nevertheless, strong species-specific ecophysiological response patterns could be identified, as well as a stimulating effect of Hütelmoor water on algal growth.

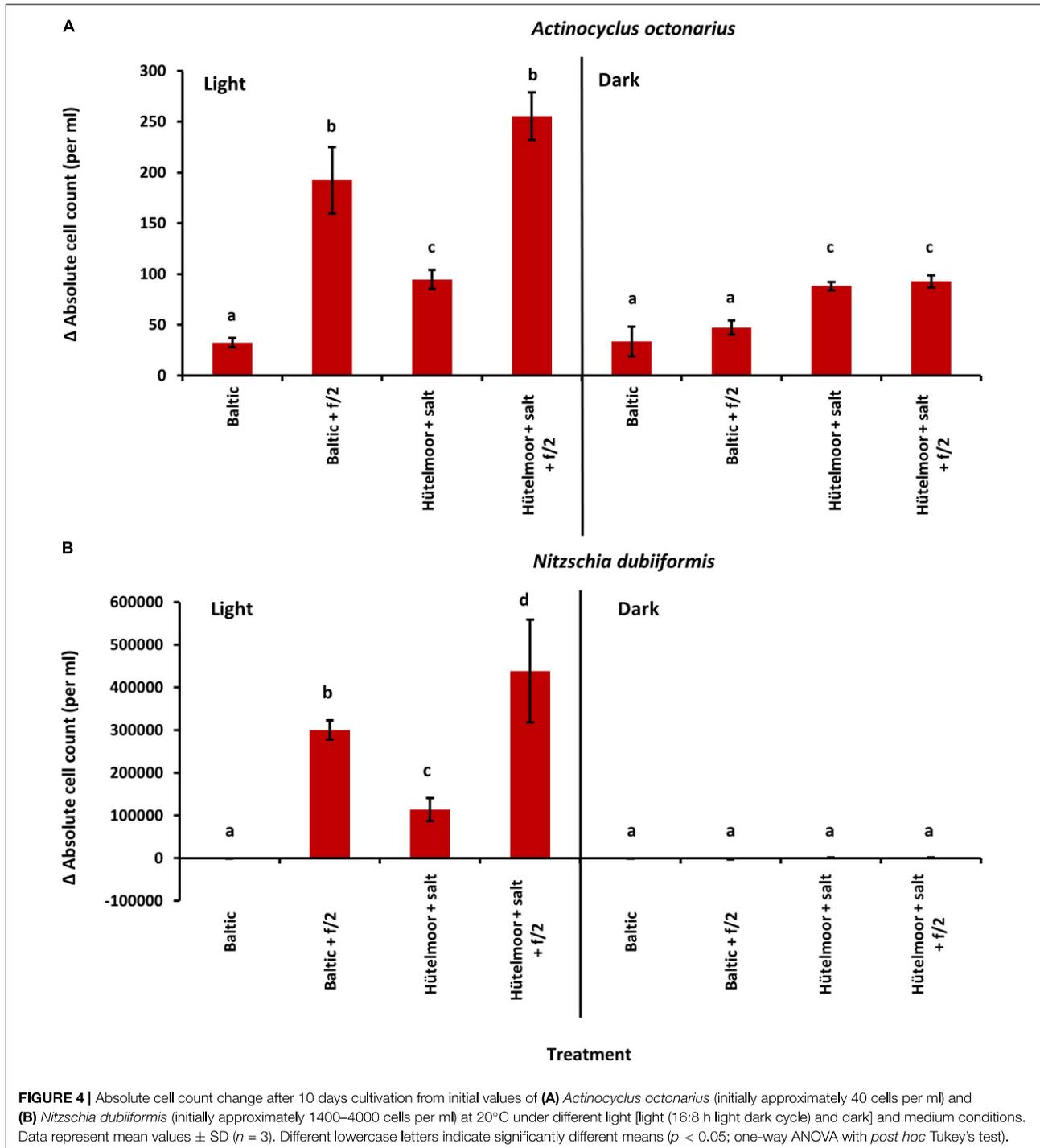
Light and Temperature Effects on Photosynthesis and Respiration

The *PE*-curves of the investigated species revealed maximum net primary production over a wide range of photon fluence rates. Similar responses were reported by Colijn and Buurt (1975) on mixed benthic diatom populations from the Dutch Wadden Sea, which were dominated by few *Navicula* species exhibiting saturated photosynthesis between 135 and 810 μmol photons m⁻² s⁻¹ at 6 and 12°C without photoinhibition. Microphytobenthic assemblages from the intertidal zone of the west coast of Portugal, including *inter alia* *Nitzschia* and *Navicula* species, were also not photoinhibited when kept under increasing photon fluence rates up to 1700 μmol photons m⁻² s⁻¹ (Serodio et al., 2006). Although the important function of benthic diatoms in primary production in the shallow non-tidal waters of the Baltic Sea has been documented on a community level under different environmental settings (Wulff et al., 1997; Meyercordt and Meyer-Reil, 1999; Meyercordt et al., 1999), few studies exist on the underlying benthic diatom ecophysiology and ecological tolerances in these brackish habitats (e.g., Woelfel et al., 2014).



Woelfel et al. (2014) carried out similar experiments, measuring the photosynthetic responses of three benthic diatoms including *M. moniliformis* and *N. perminuta*, which, however, were isolated from a protected shallow-water area (15–30 cm

water depth, 15–20 S_A) approximately 65 km west of Hütelmoor. According to this study, maximum photosynthesis in both taxa was reached at 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and no photoinhibition up 600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was found.



However, NPP_{max} and the light-saturated range at 19°C and 24°C were two times higher compared to the respective taxa of the present study. Respiration of *N. perminuta* was similar to that in Woelfel et al. (2014), while it differed in *M. moniliformis* measurements. Respirational differences might be intuitively explained by a different degree of heterotrophic bacterial

activity as cultures were not axenic. Any excretion of organic compounds by diatoms, which is known to be stimulated by high photon fluence rates, might potentially increase bacterial respiration, which would methodically lead to overall higher oxygen consumption signals (Epping and Jørgensen, 1996; Bohórquez et al., 2018). However, in the present study all diatom

cultures showed always very low numbers of associated bacteria ranging from 0.05 to 0.2% of the diatom biomass, and hence we conclude that the bacterial influence on the oxygen signals can be neglected. Wolfstein and Stal (2002) found in a similar culture approach that bacterial biomass accounted for only 0.04% of the algal carbon and hence did not influence photosynthetic ^{14}C -fixation experiments. In addition, the exudation of organic compounds by benthic diatoms such as *Cylindrotheca closterium* typically occurs after longer exposure to high photon fluence rates above $500\text{--}1000\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$, as a result of overflow metabolism due to limited internal storage capacity (Wolfstein and Stal, 2002). In contrast, all our photosynthesis experiments were rather short and during temperature treatment the maximum photon fluence rate did not exceed $270\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$, and hence C exudation seems unlikely.

An alternative explanation for the strong differences in ecophysiological response patterns among isolates of the same species might be related to ecotypic differentiation, which is considered as a genetically distinct geographic variety or population within a species that is adapted to specific environmental conditions. Ecotypic differentiation has been described for diatoms (e.g., Bailleul et al., 2010), but the designation of populations of a species as ecotypes (*sensu* Turesson, 1922) remains difficult. Differential physiological responses and a high degree of genetic heterogeneity among seasonally separated isolates of the pelagic diatom *Skeletonema marinoi* was documented and interpreted as genetic (ecotypic) differentiation of populations succeeding each other (Saravanan and Godhe, 2010). High alpha values in combination with low light compensation points between 7 and $45\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$ for all eight isolates indicate high photosynthetic efficiency under low light conditions. Different abiotic factors, such as clouds and turbidity, decrease light availability in the shallow water habitat of the coastal Baltic Sea. Decreasing photon fluence rates typically induce an increase in the amount of photosynthetic pigments such as Chl *a*, Chl *c* and fucoxanthin (Falkowski and Owens, 1980), which enhance photosynthesis under low irradiances. Although, in numerous macroalgae low-light adaptation of photosynthesis is usually coupled to strong photoinhibition under higher photon fluence densities (Hanelt et al., 1997; Bischof et al., 1998; and references therein), no indication of such expected photoinhibition could be noted in the eight benthic diatoms investigated, which may be interpreted as high photo-physiological plasticity. This high physiological plasticity can be linked to the diatom evolution. Photosynthesis in diatoms occurs in chloroplasts, which are endosymbiotic organelles derived from cyanobacteria. Since these eukaryotes acquired photosynthesis via endosymbiosis of another eukaryotic alga that already had plastids, the resulting organisms are chimeras with major genomic contributions from two or even more sources (Delwiche, 2007). As a consequence of this genomic mixing the diatom lineage with specific and often unique physiological and biochemical properties evolved. Different species of diatoms are characterized by a complex combination of genes and metabolic pathways acquired from a variety of sources such as red algae, green algae, a chlamydial parasite and bacteria (Armbrust, 2009). The consequences of this genetic mixture are

reflected in specific biochemical capabilities, which might be even species-specific.

Diatoms are well known to adjust quickly to fluctuating light regimes, such as those found in the Baltic Sea (Wagner et al., 2006; Lavaud et al., 2007). However, excessive light can lead to photodamage due to the formation of reactive oxygen species (ROS) (Apel and Hirt, 2004; Choudhury et al., 2017), causing damage of biomolecules involved in the photosynthetic machinery.

To minimize such damage on a short-term scale, benthic diatoms use two main mechanisms: (1) vertical movement in the sediment (restricted to pennate diatoms) (Perkins et al., 2010) and (2) dissipation of excessively absorbed energy through photoprotection (Nymark et al., 2009 and references therein). Using the first strategy, diatoms can escape excessive light conditions by moving downward into the sediment (Cohn and Disparti, 1994; Mouget et al., 2008; Perkins et al., 2010). This motility allows the pennate raphid species from this study (*Halamphora* sp. 1, *Halamphora* sp. 2, *N. perminuta*, *N. phyllepta*, *N. dubiiformis*, and *N. pusilla*) to avoid high light exposure. Vertical movement in nature is efficient in terms of energy requirements for protection (Perkins et al., 2010). In the present study, however, vertical movement was prevented by constant stirring of the cultures during experimentation, and hence this protective mechanism cannot explain the lack of photodamage. As no or only very little photoinhibition was detected in each diatom species at even high photon fluence rates of up to $1250\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$, it is likely that these protists used rather the second mechanism involving molecular structural change. One of the most important protection mechanisms in diatoms is the dissipation of excessive excitation energy as heat in the light-harvesting complexes of the photosystems (Goss and Lepetit, 2015). This process requires a structural change of the antenna complexes that are typically optimized with regard to efficient light-harvesting. In addition, the conversion of xanthophyll diadinoxanthin to diatoxanthin during irradiation and the reverse reaction in darkness is involved in photoprotection in diatoms (Lavaud et al., 2007; Bojko et al., 2019). The combination of genotypic differentiation and adaptive mechanisms of benthic diatoms might explain the high photo-physiological tolerance necessary to cope with strongly fluctuating light conditions in the shallow coastal water zone (Falkowski and Owens, 1980; Barrett and Schluter, 2008). In the shallow water zone of the Baltic Sea, the underwater light conditions are highly dynamic and are regularly changing because of meteorology, waves and currents, neglecting differently distributed diatom species in the depth of $3\text{--}6.5$ m in this area. The 1% depth for incident PAR has been estimated at 6.24 m at a comparable station (Zingst, 45 km further east) (Sagert and Schubert, 1999), i.e., that during summer c. $20\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$ reach the sea floor at $6\text{--}7$ m depth.

Besides light also temperature controls photosynthesis in diatoms via numerous photosynthetic enzymes, such as Ribulose-1,5-bisphosphat carboxylase-oxygenase (RuBisCO) (Tcherkez et al., 2006). At higher temperatures, the affinity of RuBisCO to CO_2 decreases compared with the affinity to oxygen, resulting in a lower carboxylase and a higher oxygenase

activity, respectively (Young et al., 2014). The seasonal water temperature in the southern coastal Baltic Sea varies from 1.3°C in winter up to 21.4°C in summer (data for 2016, H. Lippert, unpublished). Experimental temperatures covered most of the natural conditions of the Baltic Sea as well as higher extremes to identify the upper thermal tolerance of the benthic diatoms. For seven of the isolates the optimal temperature for photosynthesis was within the range of their natural habitat (Figure 3), and most species could even well cope with temperatures up to 30°C. Although heat waves in the Baltic Sea are predicted to increase (Reusch et al., 2018), the investigated benthic diatoms will probably face no physiological problems as eurythermal organisms. Temperatures > 30°C, however, led to strong or even complete inhibition of photosynthesis. Increasing temperatures are directly linked to faster metabolic rates, resulting finally in an acceleration of RuBisCO deactivation (Crafts-Brandner and Salvucci, 2000). In addition, RuBisCO is adapted to the organism's thermal environment. With rising temperature, this key enzyme is losing specificity for CO₂ and O₂, thereby getting less efficient (Tcherkez et al., 2006). Mortain-Bertrand et al. (1988) reported a seven-fold increase of RuBisCO activity of the diatom *Skeletonema costatum* at 3°C compared to 18°C, when grown for 3 months under these temperatures. Heat stress within the photosynthetic apparatus can cause instability of the photosystem II (PSII) during photosynthetic activity (Allakhverdiev et al., 2008). Even though moderate heat stress does not seriously damage the PSII, it can cause inhibition of repair mechanisms protecting the PSII (Allakhverdiev et al., 2008). Therefore, under heat stress the balance between damage and recovery processes as basis for acclimation gets disturbed (Adir et al., 2003; Mohanty et al., 2007; Murata et al., 2007), but also all other biochemical processes involved in photosynthesis. The effect of increasing temperatures on photosynthetic oxygen production and respiratory oxygen consumption in the eight benthic diatom strains showed strong differences in the temperature requirements of both physiological processes. This is not unusual since various authors clearly documented differential temperature-dependence of respiration and photosynthesis for different terrestrial and marine organisms (Allen et al., 2005; López-Urrutia et al., 2006). While Allen et al. (2005) reported a 16-fold increase in respiration over the temperature range 0–30°C due to the temperature dependence of ATP synthesis in respiratory complexes, only a fourfold increase in photosynthesis over the same temperature gradient occurred because of the temperature dependence of RuBisCO. While most diatom isolates exhibited optimum photosynthesis between 10 and 30°C, respiration was highest between 20 and 40°C. In addition, photosynthesis under lower temperatures was generally more efficiently functioning than respiration, while the opposite was true for higher temperatures, where respiration typically had enhanced activity rates compared to photosynthesis. The application of the model of Blanchard et al. (1996) specifies these findings. This is in accordance to Hancke and Glud (2004), who found for microphytobenthic communities exponentially increasing respiration rates with rising temperatures but without a pronounced optimum. Higher or lower temperatures than

those below the 20% percentile will best reflect thermal stress (Eggert et al., 2003).

The comparison of the calculated activation energy (E_a) between photosynthesis and respiration of the benthic Baltic Sea diatoms studied also indicates different temperature requirements for photosynthesis and respiration. However, a closer look on the data (Table 4) shows a slightly heterogeneous response pattern, as 5 out of 8 species confirm lower temperature requirements for photosynthesis than for respiration (Karsten et al., 2014; Padfield et al., 2016; Pierangelini et al., 2019), while the remaining 3 taxa do not follow this trend. Additionally, using a very similar methodological approach, terrestrial microalgal species studied by Pierangelini et al. (2019) exhibited slightly lower activation energies for both respiratory and photosynthetic processes, thereby allowing the conclusion that metabolic rates in benthic diatoms are activated faster than those in terrestrial microalgae under increasing temperatures.

Metabolic rates of phytoplankton are increased by rising temperature (Gillooly et al., 2001; Brown et al., 2004). These metabolic rates, however, are also dependent on nutrient supply. Maranon et al. (2018) reported that nutrient limitation besides temperature is a major component that has a suppressing influence on the activation energy for metabolic processes. These authors provided an explanation in the carbon fixation mechanism, that can be maintained under lower temperatures due to the abundance and specificity of the carbon fixing enzyme RuBisCO (Maranon et al., 2018), and as already discussed above. Measurements of photosynthetic and respiratory responses as function of increasing temperature also reflect species-specific activation energies for the thermally decoupled photosynthesis and respiration. Even though both processes are not yet fully understood in benthic diatoms of the Baltic Sea, it is assumed that these different thermal requirements allow rapid acclimation to changes in fluctuating temperature conditions, which are typical for shallow water habitats (Jurasinski et al., 2018), thereby maintaining carbon transfer for efficient cell growth (Padfield et al., 2016; Pierangelini et al., 2019).

The conspicuously different temperature requirements for photosynthesis and respiration can be explained by the fact that the first process is more dependent on light-related processes (light-absorption, energy transfer etc.) than on temperature, while the second one is light-independent and thus mainly controlled by temperature (Atkin and Tjoelker, 2003). Diatom respiration consists of a set of catabolic reactions, localized in different cellular compartments and controlled by a set of specific enzymes, of which many exhibit different temperature optima. In case of the Baltic Sea species the measured temperature optima lie in the range of values typical for organisms from the temperate to warm-temperate region.

Comparing the PE-curve data (Figure 1) with those from the temperature experiment (Figure 2) indicate some inconsistencies between the maximum photosynthetic rates (NPP_{max}) at 20°C. While in *M. moniliformis*, *Halamphora* sp. 1, *N. phyllepta*, and *N. pusilla* both experimental treatments led to almost identical NPP_{max} values, in *A. octonarius* and *N. perminuta* NPP_{max} rates were 1.7–2.5 fold higher in the PE-curve experiment. In contrast in *Halamphora* sp. 2 and *N. dubiiformis* NPP_{max} values

were 2.2–3.5 fold higher in the temperature experiment. These data indicate that the temperature effect on photosynthesis and respiration is species-dependent, and that the diatom strains respond differently to the experimental design. All cultures were kept and exponentially grew at 20°C, and hence the *PE*-curve data (recorded at 20°C) reflect long-term acclimation to this temperature. In contrast the temperature experiment included rather drastic temperature changes over short time intervals, which are unusual in the natural habitat, but provide important information on the ecophysiological performance, plasticity and tolerance width of each species. The most important conclusion which can be drawn from the data presented is, that diatoms of a microphytobenthic community exhibit an array of specific temperature tolerances from stenothermal to eurythermal response patterns, which might explain the ecological success of such sedimentary phototrophic biofilms under fluctuating temperature conditions in shallow coastal water habitats all over the world (Cahoon, 1999). The underlying mechanisms are unstudied, but might be explained by species-specific temperature-induced difference in biochemical responses as discussed above.

The Effect of Hütelmoor Water Components on Growth

Coastal areas are under high environmental pressure due to natural dynamics from the terrestrial and the marine sites as well as due to anthropogenic effects such as construction of coastal protection measures and global change. Some of these areas, such as the Hütelmoor, are now undergoing restoration forcing an exchange between land and sea processes and fluxes (Jurasiński et al., 2018).

Export of nutrients and organic compounds from the coastal peatland into the adjacent shallow water zone can be expected, which might stimulate growth of benthic diatoms and other microorganisms. Indeed, the Hütelmoor water was strongly enriched with all essential nutrients compared to Baltic Sea water. The growth assays with *N. dubiiformis* and *A. octonarius* experimentally prove for the first time, that peatland water has a stimulating effect on growth in the light, which can be explained by the higher nutrient concentrations in the Hütelmoor water media. In addition, while *N. dubiiformis* did not grow in the dark, growth of *A. octonarius* occurred in all media when kept in the dark. However, the significantly higher growth of *A. octonarius* in Hütelmoor compared to Baltic Sea media in the dark, cannot be explained by higher nutrient values. Measurements of the organic carbon revealed about 20 times higher organic content in Hütelmoor based media pointing to organic compounds which might support heterotrophic growth under dark conditions. Heterotrophy in diatoms is a well described metabolic capability, for example, in diatoms living inside and underneath the sea ice in the polar regions, or when buried in sediments (Tuchman et al., 2006; Morales-Sánchez et al., 2014). A wide range of carbon sources for heterotrophic metabolism in phototrophs have been identified including acetate, lactate and glucose (Tuchman et al., 2006; Wang et al., 2012).

Heterotrophic capabilities seem to be an ecological advantage for benthic diatoms in shallow water zones, because

hydrodynamic and meteorological conditions at the southern Baltic Sea coast are characterized by strong wind, waves and currents causing regular sediment resuspension. Under these rather instable conditions benthic diatoms, which are often strongly attached to sand grains (K. Kuriyama, personal communication) get regularly buried, and the switch from a phototrophic to a heterotrophic mode might contribute to survival. Polar benthic diatoms can survive several months of darkness by utilization of internal storage compounds, such as the lipid compound triacylglycerol (Schaub et al., 2017). To our knowledge, no other study indicating stimulating effect of coastal peatland water on growth of nearshore benthic diatoms has been published. Therefore, more experimental investigations are needed to better understand biogeochemical processes and their ecological consequences across the land-sea interface.

CONCLUSION

Overall, the studied benthic diatom strains exhibited a high photo-physiological plasticity under increasing photon fluence rates (0–1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), along with broad maximum net primary production rates and lack of pronounced photoinhibition. In addition, these diatoms can be characterized as eurythermal organisms. All these ecophysiological data indicate a high tolerance of benthic diatoms against the fluctuating environmental conditions in the shallow water coastal zone of the southern Baltic Sea. In addition, first experimental results point to heterotrophic capabilities, at least in some strains, which is fueled by organic coastal peatland compounds. These data document the importance of follow-up studies to better characterize and understand exchange processes and their ecological consequences along terrestrial-marine gradients.

DATA AVAILABILITY

Upon acceptance of the article all raw data will be published on PANGAEA and National Center for Biotechnology Information (NCBI) website.

AUTHOR CONTRIBUTIONS

AG, SG-P, and UK developed the idea and elaborated the concept. LP, AG, SG-P, VS, and KK provided experimental or taxonomic data. All authors organized and conducted the data analyses. LP, AG, and SG-P wrote the first draft of the manuscript, which was commented and edited by all other authors.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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4.2 Publication II

Ecophysiological and Cell Biological Traits of Benthic Diatoms from Coastal Wetlands of the Southern Baltic Sea

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The German Baltic Sea coastline is characterized by sea-land transition zones, specifically coastal peatlands. Such transition zones exhibit highly fluctuating environmental parameters and dynamic gradients that affect physiological processes of inhabiting organisms such as microphytobenthic communities. In the present study four representative and abundant benthic diatom strains [*Melosira nummuloides*, *Nitzschia filiformis*, *Planothidium* sp. (st. 1) and *Planothidium* sp. (st.2)] were isolated from a Baltic Sea beach and three peatlands that are irregularly affected by Baltic Sea water intrusion. Ecophysiological and cell biological traits of the strains were investigated for the first time as function of light, temperature and salinity. The four strains exhibited euryhaline growth over a range of 1–39 S_A, surpassing *in situ* salinity of the respective brackish habitats. Furthermore, they showed eurythermal growth over a temperature range from 5 to 30°C with an optimum temperature between 15 and 20°C. Growth rates did not exhibit any differences between the peatland and Baltic Sea strains. The photosynthetic temperature optimum of the peatland diatom isolates, however, was much higher (20–35°C) compared to the Baltic Sea one (10°C). All strains exhibited light saturation points ranging between 29.8 and 72.6 μmol photons m⁻² s⁻¹. The lipid content did not change in response to the tested abiotic factors. All data point to wide physiological tolerances in these benthic diatoms along the respective sea-land transition zones. This study could serve as a baseline for future studies on microphytobenthic communities and their key functions, like primary production, under fluctuating environmental stressors along terrestrial-marine gradients.

Keywords: growth rate, photosynthesis, respiration, salinity, temperature, lipids, peatland, PDMPPO

INTRODUCTION

The Baltic Sea is almost completely surrounded by land masses and the tidal range is small (Jurasiński et al., 2018). Nevertheless, wind and atmospheric pressure can cause strong waves and changes in water levels including storm floods (Lass and Magaard, 1996; Karsten et al., 2012). The lasting sea level rise is strengthened by the isostatic subsidence of the southern Baltic Sea coastline (Johansson et al., 2014), whereby the shoreline continuously recedes over time (Harff et al., 2017).

Due to this decline, exchange processes between the Baltic Sea and the proximate land will increase in the future facilitated by sea level rise (Jurasinski et al., 2018). Strong gradients in light climate, temperature and salinity across the sea-land transition zone might be the consequence, which in turn affect all inhabiting biota, for example, benthic microalgae (Karsten et al., 2012).

Wide shallow ecosystems are common for low-lying coastal areas of the southern Baltic Sea (Jurasinski et al., 2018) which are sensitive to sea-level rise. Peatlands, such as the nature reserve “Heiligensee und Hütelmoor” (in brief and in the following “Hütelmoor”), often constitute the sea-land transition zone (Jurasinski et al., 2018). The Hütelmoor is located near Rostock, Mecklenburg-Western Pomerania, north-eastern Germany. Like most peatlands in northern Germany, it has been artificially drained with the intensification of anthropogenic land use in the last centuries (Jurasinski et al., 2018). Today nature conservation has been given priority and the Hütelmoor is part of a restoration project, which aims to recreate the original conditions as a coastal peatland by introduced rewetting. Flood protection measures like dunes, which separate this peatland from the Baltic Sea, are not maintained. In the last years two storm surges occurred leading to a massive Baltic Sea water inflow with subsurface saltwater intrusion into the Hütelmoor caused by a break of the main dune.

Besides this natural rewetting of a coastal peatland, other wetlands in north-east Germany are currently reconstructed by fostering their connection to the adjacent Baltic Sea. The polder Drammendorf on the island Rügen, Germany had been cut off from the lagoon Kubitzer Bodden for over 100 years and was rewetted in November 2019 by a dyke removal. This resulted in an inflow of water from the Kubitzer Bodden due to its lower terrain height (Janssen et al., 2019).

While these active rewetting processes will increase in the future, some peatlands have been part of restoration projects for decades. As part of the nature reserve “Insel Koos, Kooser See und Wampener Riff” the previously drained coastal peatland “Karrenderfer Wiesen” near Greifswald, Germany has been under a rewetting process since 1993 after a dyke deconstruction (Seiberling et al., 2008). This has led to sporadic and periodical flooding by the Baltic Sea of low-lying areas of the Karrenderfer Wiesen (Janssen et al., 2019).

At all these sites the physico-chemical conditions are drastically changing on short time scales leading to strong gradients and highly dynamic diurnal and seasonal fluctuations, with strong influence on all benthic organisms in the coastal shallow water zone and the adjacent peatlands. Saltwater intrusion due to sea-land exchange processes may influence the biodiversity and distribution as well as the physiological performance of microphytobenthic assemblages by causing sudden and strong changes in salinity. The recent Hütelmoor flooding events created a horizontal salt gradient as salinity increased directly behind the dune with a gradual decline with more inland distance (Jurasinski et al., 2018).

Microphytobenthic communities are typically dominated by mostly pennate diatom species, which strongly contribute to the primary production and act as a filter for nutrients and other fluxes at the water-sediment-interface (Risgaard-Petersen et al., 1994; Falkowski et al., 1998; Cahoon, 1999). Benthic diatoms

are vitally involved in the carbon-, nitrogen-, phosphorus- and silicate-cycling in shallow coastal waters (Morel and Price, 2003; Wilhelm et al., 2006). Their biomass is important for carbon, energy and nutrient supply for the marine food web and serve as the main food source for benthic grazers (Cahoon, 1999; Sackett et al., 2016). Therefore, benthic diatoms can be considered as one of the ecologically most important and successful protist groups in shallow coastal waters (Wilhelm et al., 2006).

Growth is the most relevant of all physiological processes in reflecting the fitness of an organism, as it integrates all environmental effects and reflects the acclimation potential. One of the main key features of diatoms is their amorphous silica cell wall, the frustule (Brinkmann et al., 2011). The complex and energy-intensive process of the formation of the frustule can be disrupted by many environmental factors like nutrient deficiency, temperature, and salinity (Davis et al., 2005; Roubeix and Lancelot, 2008; Javaheri et al., 2015; Ma et al., 2019; Petrou et al., 2019). The impact of different abiotic factors, like salinity and temperature, on growth of benthic diatoms from the land-sea interface of the Baltic Sea is almost unstudied so far (Woelfel et al., 2014). Benthic diatoms from the southern Baltic Sea are euryhaline, as they can grow from nearly freshwater up to hypersaline conditions (1–50 S_A). However, they revealed the highest growth in brackish water (15 S_A) with rates of 0.8–1.4 $\mu\text{ d}^{-1}$ (Woelfel et al., 2014). Likewise, benthic estuarine diatoms showed a broad tolerance range as they grow in salinities ranging from 9.5 to 32 S_A while species isolated from freshwater exhibited impaired growth at higher salinities (Trobajo et al., 2011; Glaser and Karsten, 2020). Similar data on benthic diatoms from the Hütelmoor or other peatlands are missing.

Temperature is another important driver for biotic activity as temperature controls the activity of photosynthetic enzymes (Tcherkez et al., 2006). In the shallow southern Baltic Sea close to the beach temperature seasonally fluctuates from 4°C in winter up to 22°C in summer (Lippert et al., 2017). Previous studies of coastal diatom species showed that most of the investigated species are eurythermal, as there is no or only minor temperature influence on the growth rates between 7 and 27°C (Woelfel et al., 2014). Only very low (4°C) or very high (40°C) temperatures strongly reduced the growth rates of benthic diatoms (Scholz and Liebezeit, 2012). Few studies have been undertaken so far on the influence of temperature on photosynthesis and respiration of benthic diatoms in the Baltic Sea (Woelfel et al., 2014; Prelle et al., 2019). The data of these authors indicate at least 20% photosynthetic efficiency from 5 to 35°C, confirming eurythermal traits.

As a response toward stress, raphid, benthic diatoms can move vertically and horizontally in and on top of sediments (Hillebrand and Sommer, 1997; Wasmund and Uhlig, 2003; Blommaert et al., 2018) by excreting extracellular polymeric substances (EPS) (Beninger et al., 2018).

Diatoms produce and accumulate oil mainly in the form of triacylglycerols (TAGs), which are sequestered in lipid droplets (LDs) in the cytoplasm. The biosynthesis of TAGs is controlled by temperature, light conditions, and salinity (for review see Li-Beisson et al., 2019). Diatoms produce up to 60% of their cellular mass as TAGs (Sheehan et al., 1998). Under favorable conditions

only small amounts of these neutral lipids are enriched, while under stress LDs are rapidly formed in high amounts (in general 20–50% of cell dry weight) (Hu et al., 2008). During stress recovery, LDs are remobilized serving as an energy source. Stress leads to an imbalance in energy and redox homeostasis, which must be actively counteracted under energy consumption. In diatoms, lipid accumulation seems to be more relevant as energy rather than as carbon storage (Hu et al., 2008). A large portion of cellular carbon is redirected into TAG metabolism under stressful conditions (Alipanah et al., 2015), leading to an increase in oil synthesis while growth and biomass production are slowed down. Consequently, a higher LD production rate goes ahead with a deprivation in overall productivity of the cell (Sheehan et al., 1998; Meksiarun et al., 2015).

As intact peatlands have been proven to be beneficial as carbon sink and nutrient buffering, renaturation projects have increased along the Baltic Sea coast (Vasander et al., 2003). However, the impact of renaturation and changes of abiotic parameters on microphytobenthic processes have not yet been investigated, despite their strong involvement in ecological processes such as primary production. Here we are investigating the ecophysiological response and cell biological traits of four isolated diatoms strains from the sea-land transition zone to the parameters light availability, temperature, and salinity that highly fluctuating in renaturing peatland systems, addressing the following hypotheses: (I) The peatland diatom strains grow and photosynthesize in a wider temperature range than found in their originating habitat due to their high physiological plasticity. (II) The peatland isolates have low-light requirements for photosynthesis as they are regularly confronted with elevated turbidity. (III) The peatland diatom strains exhibit a broad salinity tolerance because of recurring floods of the Baltic Sea, which cause an increased salinity gradient in the coastal peatlands.

MATERIALS AND METHODS

Study Site

Undisturbed sediment cores (diameter: 5 cm; length: 10 cm) taken in May/June 2019 from four study sites (**Figure 1**) were used for benthic diatom isolation. Two sediment cores originated from the nature reserve “Heiligensee und Hütelmoor” northeast of Rostock, Mecklenburg Pomerania, Germany. The first core was taken on a sandy beach side (54.22550N, 12.17185E) in close vicinity to the Baltic Sea (**Figure 2A**). Salinity, temperature, and water availability highly fluctuate, as the sampling point gets flooded irregularly with Baltic Sea water. The second core was taken within the coastal peatland (54.21212N, 12.18343E) at a water depth of 20–30 cm (**Figure 2B**). Water temperature ranged from ~4°C in winter to ~26°C in summer (2019), while salinity was relatively stable between 2.5 and 5 S_A (2019). The third sampling core was taken from the peatland Karrendorf (**Figure 2C**), in a small trench at 20–30 cm water depth (54.15796N, 13.38859E). In winter, water temperature ranged around 5°C and salinity at 6–7 S_A (2019). There are no data available on highest water temperature in summer for this

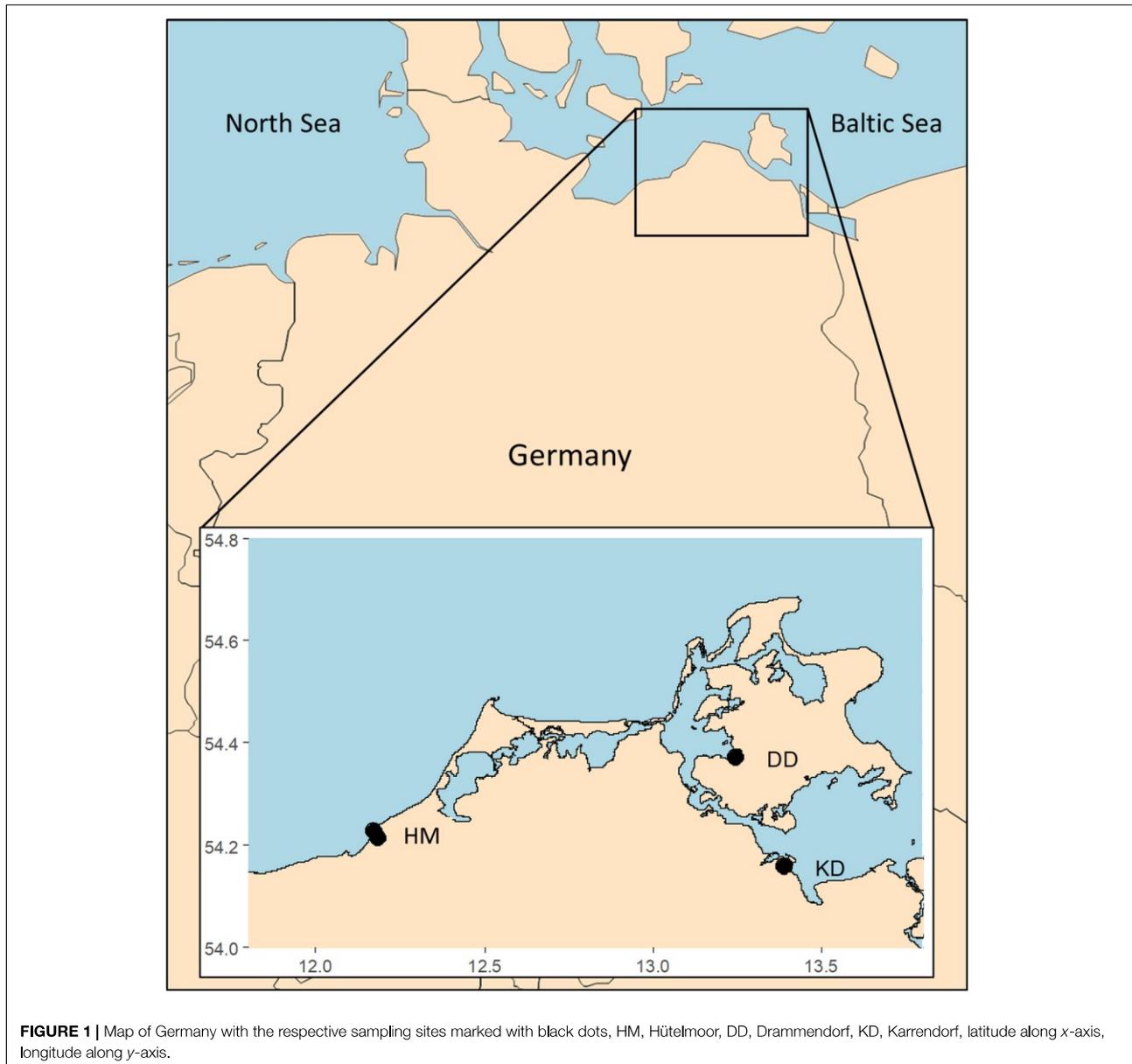
sampling point as it fell dry. Measurements of a similar sampling point about 100 m west (54.15781N, 13.39241E) indicate water temperature of ~30°C and salinities of up to 18 S_A in summer before falling dry. The fourth core was taken in the peatland Drammendorf (**Figure 2D**) from a small trench at about 20–30 cm water depth (54.36971N, 13.24384E). Water temperature in these trenches before flooding of this area and at the time of sampling ranged between ~4°C in winter and ~30°C in summer, and salinity ranged between 0.5 and 3.5 S_A.

Cultures Establishment and Culture Conditions

The upper 1 cm layer of each sediment core served as basis for diatom isolation. 2.3 cm³ of fresh sediment were incubated for approximately 2 weeks in Guillard’s f/2 medium (Guillard and Ryther, 1962; Guillard, 1975) enriched with metasilicate (Na₂SiO₃ · 5 H₂O; 10 g 100 ml⁻¹) to a final concentration of 0.6 mM (further referred to as culture condition). The basis culture medium was Baltic Sea water (~12 S_A) enriched with synthetic sea salt (hw Marinemix® professional, hw Wiegandt Auaristik, Krefeld, Germany) to achieve a final salinity of 15 S_A. Culture conditions were set to 20°C at 30–50 μmol photons m⁻² s⁻¹ under a 16:8 h light:dark cycle provided by Osram Daylight Lumilux Cool White lamps L36W/840 (Osram, Munich, Germany). After microscopic inspection and ca. 2–4 weeks incubation, single cell isolation was performed under sterile conditions by manual cell picking of the most abundant benthic diatoms and their repeated transfer into fresh cultivation medium until unialgal cultures were established. Isolates were maintained as clonal, but not axenic cultures—DAPI-staining [4',6'-diamidino-2-phenylindole, a blue-fluorescent DNA stain for bacteria (Carl Roth, Karlsruhe, Germany)] typically resulted in low bacteria numbers ranging in maximum of 0.05–1% of the diatom biomass. From each of these sampling sites, one representative isolate was used for the experimental set-ups. These species were later identified as: *Melosira nummuloides* C. Agardh 1824 (Hütelmoor, Beach), *Planothidium* sp. (st. 1) Round and L. Bukhtiyarova, 1996 (Hütelmoor, peatland), *Planothidium* sp. (st. 2) Round and L. Bukhtiyarova, 1996 (Karrendorf) and *Nitzschia filiformis* (W. Smith) Van Heurck 1896 (Drammendorf).

All strains were identified using morphological and molecular approaches. Morphological identification was supported using light microscopic images of living and valve cells, as well as scanning electron microscope (SEM) images. Isolates were treated in 15% H₂O₂ to remove most organic contents resulting in the detachment of the epi- and hypotheca of most of the cells. Valves were coated with an ultrathin layer of graphite by high-vacuum evaporation (Leica EM SCD 500; Fa. Leica, Bensheim) for electrical conductivity. Images were taken with a Field Emission Scanning Electron Microscope MERLIN® VP Compact (Fa. Zeiss, Oberkochen) and the SmartSEM® program (version 5.09; Fa. Zeiss, Oberkochen) at varying magnifications of 1500×–15,000×.

Recent literature was used for species identification (Hofmann et al., 2013). Identified species were compared to the data base



Algae Base¹ for recent nomenclature. Molecular analysis followed the approach of Abarca et al. (2014) using the primer Diat-rbcL-R and Diat-rbcL-iF for the *rbcL* gene. PCR mix (20 μ l) consisted of 10 μ l MyTaqTM mix (Bioline), 6 μ l HPLC H₂O, 1 μ l of each primer (20 μ M) and 2 μ l DNA sample, following the PCR regime of Abarca et al. (2014). Sanger sequencing was conducted by Microsynth Seqlab GmbH, Göttingen, Germany. All sequences were submitted to the National Center for Biotechnology Information (NCBI) under the following accession numbers: *Melosira nummuloides* (MW070612) (strain PTM9a), *Planothidium* sp. (st. 1) (MW070614) (strain PTM25),

Planothidium sp. (st. 2) (MW070611) (strain PTM7), and *Nitzschia filiformis* (MW070613) (strain PTM10). These cultures are available at the culture collection of the Department Applied Ecology and Phycology, University of Rostock.

Growth Rates

To determine growth rates of the four benthic diatoms strains in dependence of temperature and salinity, the fluorescence of chlorophyll *a* was used as proxy for biomass. *In vivo* fluorimetry is a non-invasive, simple, and robust method, which can be applied to adhering and filamentous microalgae, and is particularly suitable for benthic diatoms (Karsten et al., 1996; Gustavs et al., 2009). *In vivo* chlorophyll *a* fluorescence

¹www.algaebase.org



measurements as proxy for growth were performed with a self-constructed growth fluorimeter based on the basic electronic unit of a MFMS fluorimeter (Hansatech Instruments, King's Lynn, United Kingdom) according to the protocol of Karsten et al. (1996). Bright-blue light LEDs emission (Nichia, Nürnberg, Germany) with a peak emission wavelength at 470 nm were selected for excitation of the chlorophyll *a* fluorescence and pulsed with a modulation frequency of 870 Hz. Chlorophyll *a* fluorescence was detected as relative units by an amplified photodiode and was separated from scattered excitation light through a long pass glass filters (RG 665; Schott, Mainz, Germany) and a bright-red gelatin filter (Lee, Brussels, Belgium). *In vivo* chlorophyll *a* fluorescence units correlate very well to cell number, organic carbon and chlorophyll *a* concentration in diatoms as shown by Karsten et al. (1996) and Gustavs et al. (2009).

The cultures were grown in disposable petri dishes with cover lids in a volume of 15 ml culture medium and measured every 24 h for 10 days following the procedure of Gustavs et al. (2009). All cultures were acclimated to the new culturing conditions for 4 days before the experiments. Light was kept constant at $25 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ following a 16:8 light:dark cycle (Osram light sources see above). The cultures were kept in water baths or air-conditioned rooms to ensure constant temperature conditions, all experiments were done in triplicates. Six temperatures (5,

10, 15, 20, 25, and 30°C) were tested at a salinity of $15 S_A$, which corresponded to the standard cultivation conditions (see above). To determine the growth rate dependence on salinity, six salinities were chosen: 1, 5, 10, 15, 27, and $39 S_A$; incubation temperature was kept constant at 20°C. The salinities were adjusted using synthetic sea salt (hw-Marinemix® professional) dissolved in aqua dest. and enriched with *f/2* and metasilicate according to standard cultivation conditions (see above). Growth rates were calculated separately for every replicate using the phase, where the fluorescence signal increased exponentially, applying the following equation: $N = N_0 * e^{(\mu * dt)}$ (N —fluorescence at the measuring day, dt —difference of time in days between measuring day and starting day, μ —growth rate) (Gustavs et al., 2009).

Light Response Curves (PI-Curves)

Photosynthesis-irradiance (PI)-curves of the four diatom strains were measured according to Prelle et al. (2019). Shortly, 3 ml of thin log phase algal suspension (to avoid self-shading chlorophyll *a* content was kept at $1,270 \mu\text{g/l}$ on average) of each strain and $30 \mu\text{l}$ sodium bicarbonate (NaHCO_3 2 mM final concentration) were added to four airtight water-tempered (20°C) oxygen electrode chambers (DW1, Hansatech Instruments, King's Lynn, United Kingdom). At 10 increasing photon flux density levels ranging from 0 to $\sim 1,500 \mu\text{mol}$

photons $\text{m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR), oxygen concentration was measured using a non-invasive oxygen dipping probe (DP sensors PreSens Precision Sensing GmbH, Regensburg, Germany). Measurements consisted of a 30 min respiration phase, followed by a 10 min photosynthesis phase for each light level. The first and last minute of each measurement were excluded from the calculation. After the last measurement, chlorophyll *a* was extracted from the 3 ml algal suspension using 96% ethanol and quantified spectrophotometrically at 665 nm (Shimadzu UV-2401 PC, Kyoto, Japan) (HELCOM, 2015).

The mathematical photosynthesis model of Walsby (1997) was used for fitting and calculation of maximum rates of net primary production (NPP_{max}), respiration (R), light utilization coefficient (α), photoinhibition coefficient (β), light saturation point (I_k) and the light compensation point (I_c).

Temperature-Dependent Photosynthesis and Respiration

Following the methodological approach of Karsten et al. (2010), the photosynthetic and respiratory response of each strain at temperatures between 5 and 40°C was measured using the same oxygen optode system as for the PI-curves. After 20 min incubation in the dark, the respiratory oxygen consumption (10 min in the dark), followed by the photosynthetic oxygen production (10 min under light saturated conditions at $320 \pm 45 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR) were determined. Measurements were normalized to the total chlorophyll *a* concentration (HELCOM, 2015). Photosynthesis to respiration ratios (P:R) were calculated, excluding positive respiration and negative photosynthesis measurements.

Cell Biology

To investigate the effects of salinity and temperature on the formation of diatom frustules and the storage of lipids, the four diatom strains were incubated under different salinity and temperature conditions. Salinity effects were investigated in cultivation media of 1, 15, and 30 S_A at 20°C, temperature effects at 7, 17, and 22°C at 15 S_A (see section “Growth Rates for More Details on Cultivation Conditions”).

Cultures were pre-incubated for 4 days in disposable petri dishes with cover lids (20 ml) at the respective experimental growth conditions to acclimate and to attain log-phase. After pre-incubation, 5 ml of each culture were added into 6-well cell culture plates (Greiner BioOne) to the respective fresh medium (final volume 15 ml) and were incubated for another 7 days.

Setting the samples for the investigation of the frustule formation toward temperature and salinity, PDMPO ([2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocarbonyl)methoxy)phenyl)oxazole)] was used as stain for visualization according to Shimizu et al. (2001). A 100 $\mu\text{mol l}^{-1}$ PDMPO [LysoSensorTM Yellow/Blue DND-160” (Invitrogen, Carlsbad, United States)] working solution was prepared with DMSO (Dimethylsulfoxid) (Calbiochem, San Diego, United States) and added to each of the 6-well cell culture plate with a final concentration of 1 $\mu\text{mol l}^{-1}$. The PDMPO was incorporated into newly formed valves and visualized with an epi-fluorescence

microscope. To quantify new valve formation, fixed samples were observed at 400× total magnification using epi-fluorescence microscopes (BX 51 and IX 70, Olympus, Hamburg, Germany) under UV excitation (U-MWU, excitation: 330–385 nm, emission: > 420 nm, Olympus, Hamburg, Germany). In each sample, 400 cells were examined and categorized into: (1) at least one valve stained, (2) no valve stained.

On days 3 and 7, 1 ml culture was taken from each well and immediately fixed with glutaraldehyde (2% final concentration) and stored in the dark until further treatment.

The effects of temperature and salinity on lipid formation and lipid volume of the four diatom isolates were investigated using Nile red lipid staining according to Greenspan et al. (1985). To each 1 ml sample, 1 μl of Nile red (Carl Roth, Karlsruhe, Germany) was added. After 10 min of dark incubation, each sample was evaluated at 400-fold magnification using an epi-fluorescence microscope (BX-51, Olympus, Hamburg, Germany) under blue excitation (U-MWB, Olympus, Hamburg, Germany). Micrographs of 50 cells [*Planothidium* sp. (st. 1) and *Planothidium* sp. (st. 2)] or 20 cells (*M. nummuloides* and *N. filiformis*) per sample were taken using CellSens Standard imaging software (Olympus, Hamburg, Germany). The diameter of each lipid droplet was measured using ImageJ (Open Source by National Institutes of Health) and volume of the respective shape (oval, conical and spherical shapes) was calculated.

Statistical Analysis

All calculations and figures were made using Microsoft Office Excel (2016)—partly using the solver function by minimizing the sum of normalized squared deviations—and R (Version: 4.0.2). Statistical analysis was performed using R. Growth rates as function of temperature and temperature-dependent photosynthesis and respiration were fitted using the model of Yan and Hunt (1999), which proved to be best suitable for simple biological processes (Adams et al., 2017). Growth rates as function of salinity were not fitted due to almost no response. Confidence intervals were calculated using the library nlstools in R and are provided in **Supplementary Table 1**. Significance levels were calculated using one-way ANOVA followed by a *post hoc* Tukey’s honest significant differences test (critical $p < 0.05$).

RESULTS

Species Identification

The diatom strains were morphologically identified based on SEM images as *Melosira nummuloides* due to chain formation, as *Nitzschia filiformis* due to its characteristic genus-specific fibulae, and two *Planothidium* sp. (**Figures 3A–D**). The latter could only be identified to genus level due to unclear morphological traits for species discrimination.

Molecular analysis of the species *M. nummuloides* using NCBI BLASTn (Altschul et al., 1990) coincided to 99% with the *rbcl* gene of *Melosira nummuloides* C67 (FJ002129). Similarly, *N. filiformis* coincided to 99% with the *rbcl* gene of *Nitzschia filiformis* UTEX FD267 (HQ912453) (Theriot et al., 2010). *Planothidium* sp. (st. 1) and *Planothidium* sp.

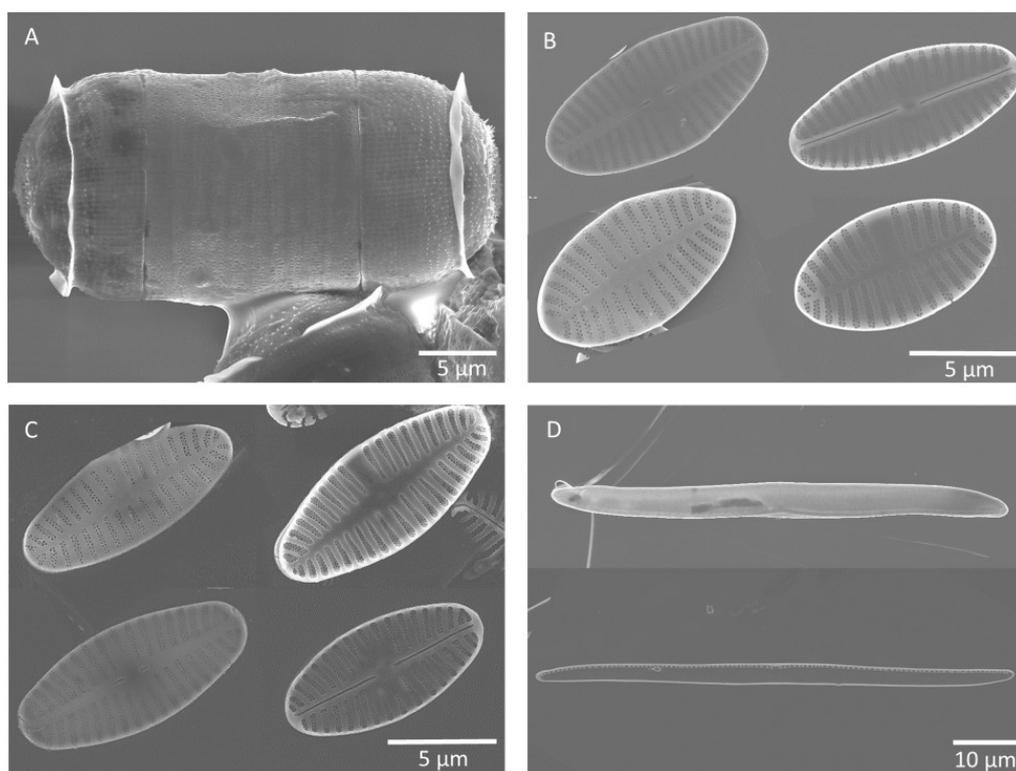


FIGURE 3 | Scanning electron microscopy images (by Kana Kuriyama) of frustules of the four benthic diatom strains investigated, **(A)** *Melosira nummuloides*, **(B)** *Planothidium* sp. (st. 1) (monoraphid—displaying raphid and araphid valve (in- and outside), **(C)** *Planothidium* sp. (st. 2) (monoraphid—displaying raphid and araphid valve (in- and outside), **(D)** *Nitzschia filiformis* (in- and outside).

(st. 2) had a 100% overlap of the *rbcl* gene sequences to each other, indicating that both strains belong to the same species. The most similar sequences were *Halamphora americana* (95%, MK045450) and *Planothidium suncheomanense* (94%, KY650831). In combination with the morphological data, we identified the two strains only to the genus level *Planothidium*.

Growth Rates

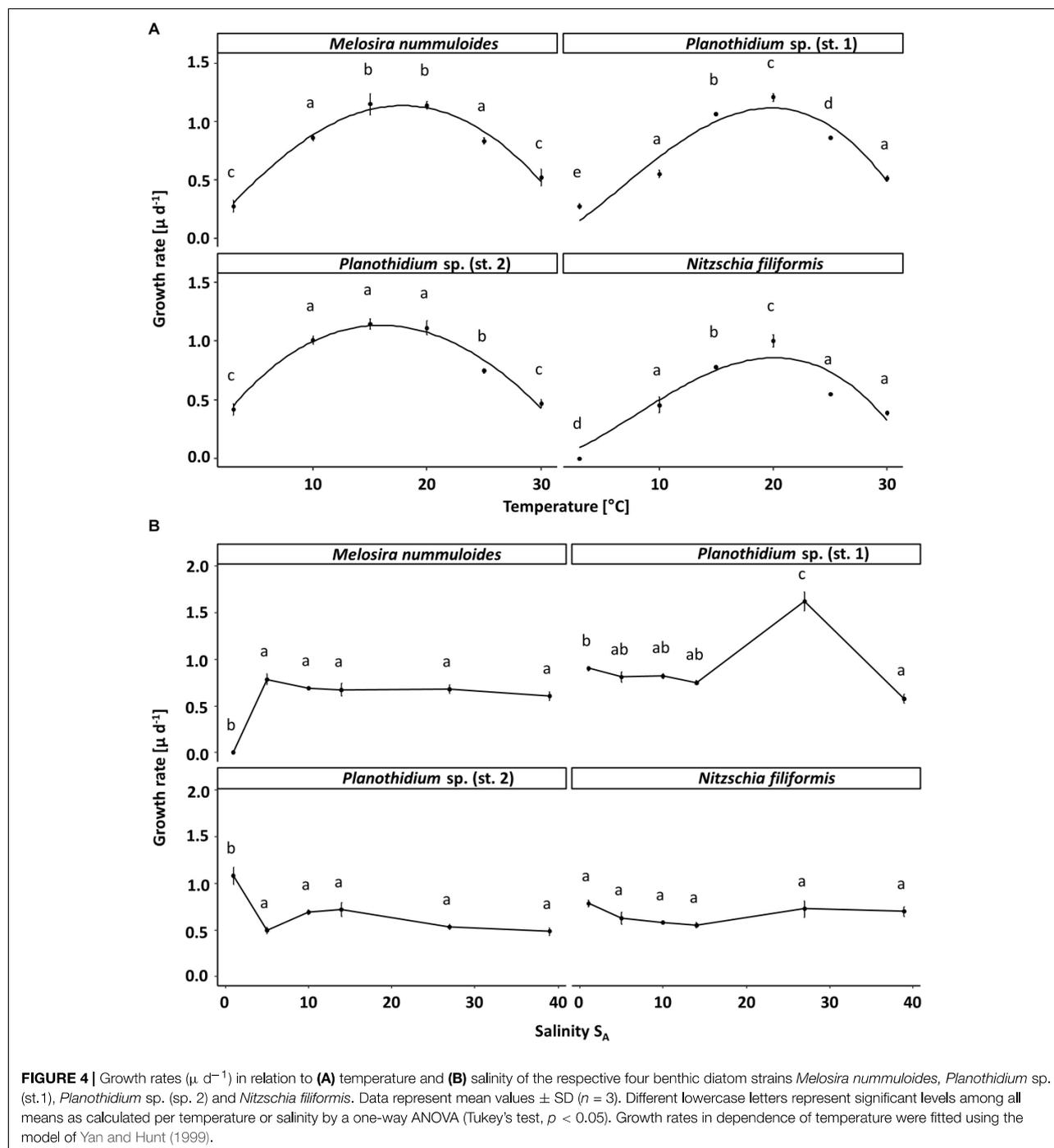
The temperature effect on the growth rates varied between the four benthic diatom strains (Figure 4). *Nitzschia filiformis* did not grow at 5°C, whereas the other strains showed growth rates up to 0.4 μ d⁻¹ at the lowest tested temperature. All strains were able to grow at the highest tested temperature of 30°C, also with growth rates at around 0.5 μ d⁻¹. The optimum temperature of *N. filiformis* and *Planothidium* sp. (st. 1) was determined at 20°C with growth rates of 0.86 and 1.11 μ d⁻¹, respectively. *Melosira nummuloides* grew best at 15 and 20°C with maximum growth rate of 1.13 μ d⁻¹, *Planothidium* sp. (st. 2) between 10 and 20°C with maximum growth rate of 1.14 μ d⁻¹ (Figure 4A). All strains exhibited a broad temperature range for optimal growth, where still 80% of the maximum growth rate is reached. *Nitzschia filiformis* and *Planothidium* sp. (st. 1) showed optimum growth between 13 and 26°C, *Planothidium* sp. (st. 1) between 8.4 and 24°C, *M. nummuloides* between 10 and 25°C.

The four tested diatom strains tolerated a broad salinity spectrum. *Planothidium* sp. (st. 2) had the highest growth with a rate of 1.1 μ d⁻¹ at 1 S_A. No significant differences between the other tested salinities were observed. The growth rate of *N. filiformis* was similar at the salinities between 1 and 39 S_A. *Melosira nummuloides* did not grow at 1 S_A but grew well with a rate of 0.8 μ d⁻¹ at all other tested salinities between 5 and 39 S_A. *Planothidium* sp. (st. 1) showed a clear optimum salinity of 27 S_A for growth (Figure 4B).

Light-Dependent Photosynthesis

Photosynthesis and respiration of the four benthic diatom strains resulted in strain-specific responses as function of increasing photon fluence rates up to ~ 1,500 μ mol photons m⁻² s⁻¹ (Figure 5). Due to low numbers of diatom-associated bacteria (<0.05–1% of diatom biomass), oxygen signals can be ascribed to the diatom response during experimentation.

Characteristic PI-curve parameters were calculated using the photosynthetic model of Walsby (1997) (Table 1). *Planothidium* sp. (st. 2) exhibited the lowest dark respiration rate (–23.75 μ mol O₂ mg⁻¹ chl *a* h⁻¹) and *N. filiformis* the highest (–48.64 μ mol O₂ mg⁻¹ chl *a* h⁻¹), with the remaining two isolates between these values. The maximum photosynthetic rates (NPP_{max}) of *Planothidium* sp. (st. 2) and *Planothidium* sp. (st. 1) were in a



similar range, while the NPP_{max} of *M. nummuloides* was only 50% of these rates and for *N. filiformis* 2.5 times higher. A similar pattern was found for the light saturation point (I_k) with *Planothidium* sp. (st. 2) exhibiting the lowest value at $36.5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and *N. filiformis* the highest value at $56.1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. The highest light utilization coefficient (α) was calculated for *N. filiformis* with $2.77 \mu\text{mol O}_2 \text{mg}^{-1} \text{chl } a$

$\text{h}^{-1} (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$. The remaining isolates had slightly lower α , ranging from 1.68 to $2.21 \mu\text{mol O}_2 \text{mg}^{-1} \text{chl } a \text{ h}^{-1} (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$. Compared to *Planothidium* sp. (st. 2) and *N. filiformis* light compensation points (I_c) were slightly higher in *M. nummuloides* and *Planothidium* sp. (st. 1), but all ranged between 14.0 and $26.1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Using the Walsby fit (1997), photoinhibition was detected in

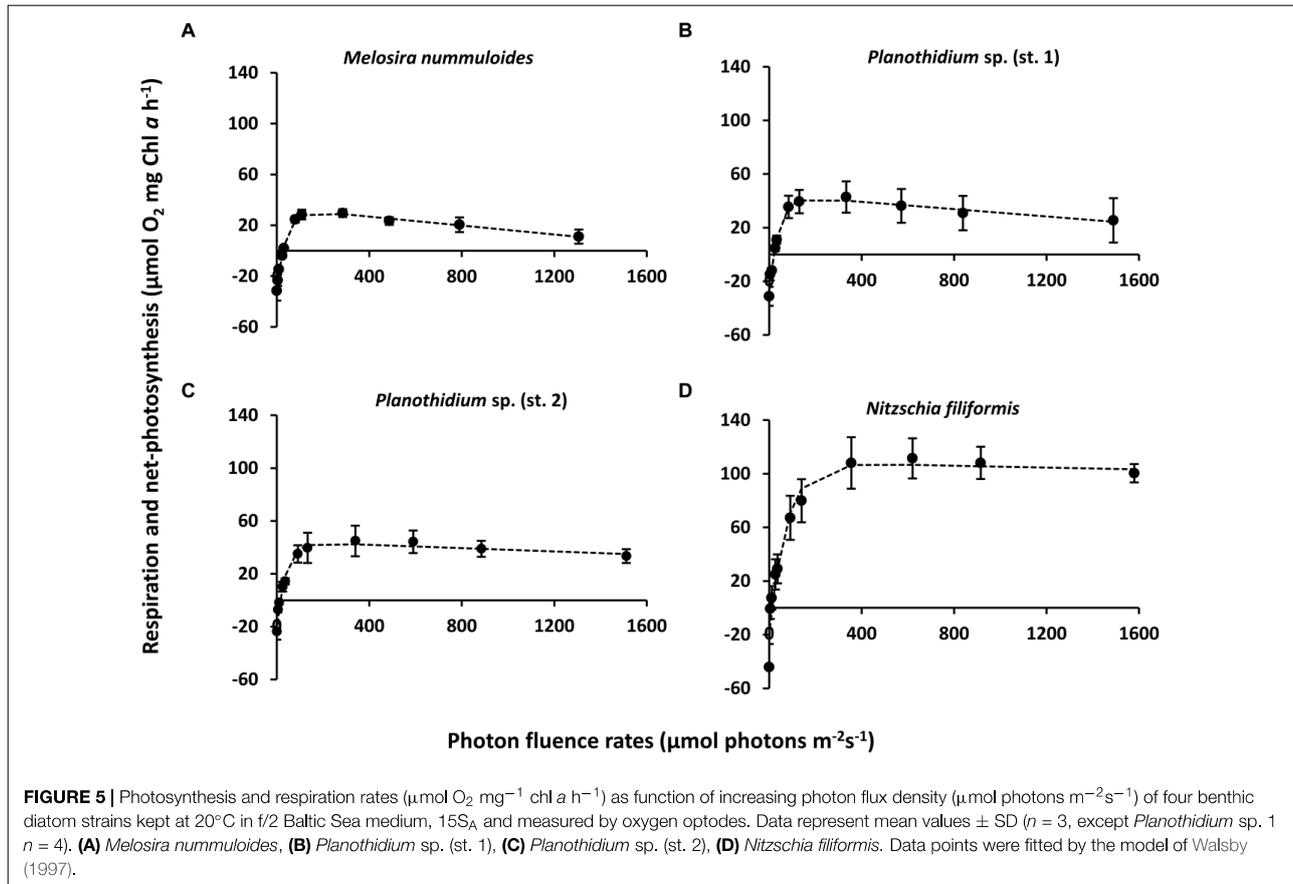


TABLE 1 | Parameter of respective *PI*-curves (Figure 5) of four benthic diatom strains [$n = 3$, except *Planothidium* sp. (st. 2) $n = 4$] kept at 20°C in a f/2 Baltic Sea medium, 15S_A .

Isolates	NPP_{max} ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl a h}^{-1}$)	Respiration ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl a h}^{-1}$)	α ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl a h}^{-1}$) ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) ⁻¹	β ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl a h}^{-1}$) ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) ⁻¹	I_k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	I_c ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	$\text{NPP}_{\text{max}}:\text{Respiration}$
<i>Melosira nummuloides</i>	30.42 ± 0.22 a	-31.42 ± 1.89 a	1.68 ± 0.72 a	-0.02 ± 0.00 a	36.66 ± 15.23 a	26.09 ± 13.44 a	0.97 ± 0.03 a
<i>Planothidium</i> sp. (st. 1)	46.09 ± 8.50 a	-31.49 ± 7.94 a	1.93 ± 0.54 a	-0.01 ± 0.01 a	40.24 ± 6.37 ab	20.95 ± 4.46 a	1.46 ± 0.57 a
<i>Planothidium</i> sp. (st. 2)	42.89 ± 10.79 a	-23.75 ± 5.96 a	1.83 ± 0.48 a	-0.01 ± 0.01 a	36.48 ± 2.91 ab	16.10 ± 1.45 a	1.81 ± 0.12 ab
<i>Nitzschia filiformis</i>	106.94 ± 17.24 b	-48.64 ± 12.89 a	1.76 ± 0.75 a	0.00 ± 0.02 a	72.60 ± 17.17 b	14.06 ± 8.21 a	5.07 ± 0.49 b

Different lowercase letters represent significant levels among all means as calculated by a one-way ANOVA (Tukey's test, $p < 0.05$). NPP_{max} represents the maximal oxygen production rate, α the initial slope of production in the light limited range, β the terminal slope of production in extensive light range (photoinhibition), I_k the light saturation point, I_c the light compensation point.

M. nummuloides and both *Planothidium* sp. cultures. NPP_{max} and respiration ratios of the four isolates ranged from 0.97 to 5.07 (Figure 5 and Table 1).

Temperature-Dependent Photosynthesis and Respiration

The four diatom strains exhibited different photosynthetic and respiratory responses to increasing temperatures between 5 and 40°C (Figure 6 and Table 2). The net photosynthetic oxygen production and respiratory oxygen consumption generally

increased with rising temperatures up to a strain-specific maximum. With further increasing temperature a decrease in photosynthetic oxygen production and respiratory oxygen consumption was observed. Highest respiration rates were measured between 30 and 40°C among all species while photosynthetic optima varied strongly from 10 to 35°C (Figure 6). The overall oxygen production of *Planothidium* sp. (st. 2) showed only weak dependence to temperature and was lowest compared to the remaining three strains with $20.0 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl a h}^{-1}$ at 25°C (Figure 6). In contrast, *N. filiformis* exhibited the overall highest photosynthetic maximum amongst

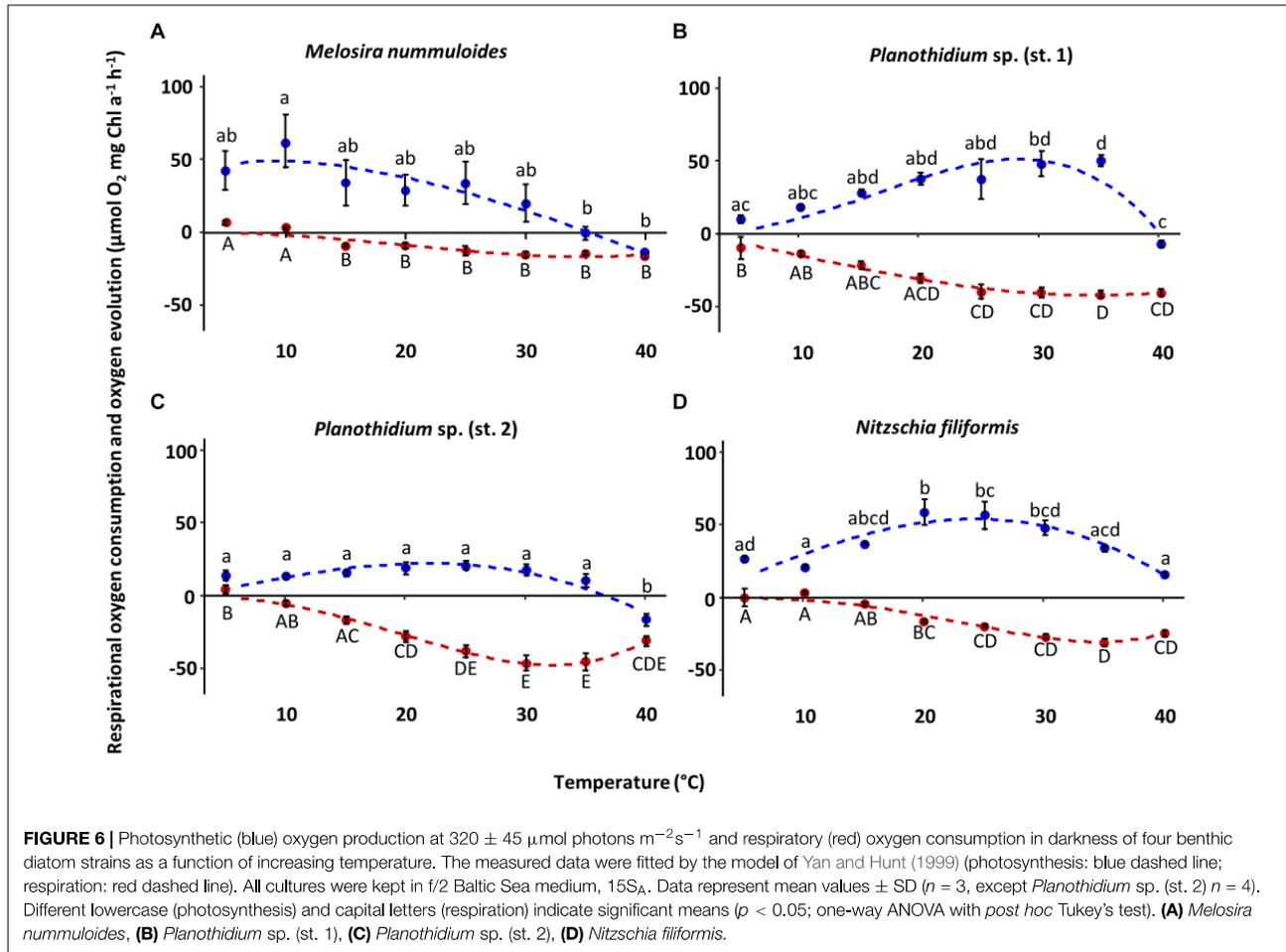


TABLE 2 | Results of model calculation for temperature-dependent growth rate, photosynthetic rate, and respirational rate following the model of Yan and Hunt (1999).

		<i>Melosira nummuloides</i>	<i>Planothidium</i> sp. (st. 1)	<i>Planothidium</i> sp. (st. 2)	<i>Nitzschia filiformis</i>
Growth	Maximal growth rate	1.1	1.1	1.1	0.9
	Optimum temperature	17.9	19.8	16.0	20.1
	Maximum temperature	33.8	33.1	33.8	32.4
	Residual sum-square	0.1	0.2	0.1	0.3
	Temperature range for				
	Optimal growth (80% growth rate)	10.4–25.1	12.9–26	8.4–23.8	13.5–26
	Growth (20% growth rate)	2.3–32.2	4.0–31.8	4.6–31.2	4.6–31.2
Photosynthesis	Maximal photosynthetic rate	49.1	51.6	22.4	54.2
	Optimum temperature	9.1	28.2	21.8	24.2
	Maximum temperature	35.3	39.9	36.6	42.7
	Residual sum-square	9,028	3,292	1,429	2,165
	Temperature range for				
	Optimal photosynthesis (80% photosynthetic rate)	2.5–19.1	21–33.9	14.1–28.8	14.9–32.7
	Photosynthesis (20% photosynthetic rate)	0.1–31.9	9.8–38.8	4.3–35.3	4–40.9
Respiration	Maximal respirational rate	-17.0	-42.2	-47.8	-30.6
	Optimum temperature	34.8	34.6	32.2	34.6
	Maximum temperature	49.1	59.4	44.5	45.4
	Residual sum-square	502.5	861.8	1,389	444.8
	Temperature range for				
	Optimal respiration (80% respirational rate)	26.2–41.7	21.9–46.1	24.6–38.2	27.6–39.9
	Respiration (20% respirational rate)	12.4–47.8	6.3–56.9	12–43.3	15.2–44.4

the four strains with $59.1 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$ at 20°C . In contrast to the other three isolates *M. nummuloides* did not follow the trend of an increasing oxygen production with increasing temperatures by reaching its maximum production of $62.8 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$ already at 10°C . Between 10 and 25°C photosynthesis decreased almost linearly (Figure 6). Modeled data of all four strains showed optimal photosynthesis (defined as 80% percentile) over a species-specific temperature span between 13 and 18°C , while reduced photosynthesis (defined as 20% percentile) was performed over a span between 29 and 37°C (Table 2).

Overall, the respiratory oxygen consumption of the four strains increased with beginning to low temperature, but decreased after reaching the strain-specific optimum, which was slightly higher than the photosynthetic optimum (Figure 6 and Table 2). In *Planothidium* sp. (st. 2) and *M. nummuloides* respiratory oxygen consumption at 5°C was not detectable, and the same was true at 10°C for *M. nummuloides* and *N. filiformis* (Figure 6). Maximum respiratory oxygen consumption was measured with $-46.9 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$ at 30°C for *Planothidium* sp. (st. 2), with $-30.3 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$ at 35°C for *N. filiformis*, with $-42.0 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$ at 35°C and with $-15.97 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$ at 40°C for *M. nummuloides* (Figure 6). The optimum respiration (80% of maximum rate) span between 22 and 40°C , while 20% of maximum respiration rate was reached at 12°C or even lower temperatures for *Planothidium* sp. st.1 (Table 2).

Calculations of P:R ratios exhibited significant differences between the strains at different temperature levels (Table 2). At 15°C , P:R ratio of *N. filiformis* was significantly higher with a value of 9.9 compared to the other temperatures. P:R ratios were otherwise rather similar (0.03–5.4) across all temperatures and strains (Table 3).

Effects of Temperature and Salinity on Diatom Frustule Formation

For each isolate, the proportion of PDMPO-stained (new) and unstained diatom valves was counted on 2 days (Figure 7). Depending on the respective growth rate derived from the chlorophyll *a* fluorescence, 1–22% of unstained cells were expected. Overall, the proportion of the newly built valves was at least 50%, even higher in most cases, at both sampling days as the exponential growth mostly occurred until day 5. Furthermore, the overall percentage of newly built valves increased slightly from day 3 to day 7, for both temperature and salinity (Figure 8). *Planothidium* sp. (st. 1) and *Planothidium* sp. (st. 2) (Figure 8) exhibited no major differences in the formation of new valves concerning both sampling days as well as varying temperatures. For *M. nummuloides*, a higher proportion of unstained valves was found at temperatures of 7 and 22°C from days 3 to 7 and a decrease was found for 17°C . At day 3, *N. filiformis* exhibited the overall lowest percentage of unstained valves at 7 and 17°C . At day 7 percentage of newly formed valves strongly increased (Figure 8).

Different salinities as well as the sampling day had almost no effect on the formation of new valves for *N. filiformis*,

Planothidium sp. (st. 1) and *Planothidium* sp. (st. 2) (Figure 8). *Melosira nummuloides* exhibited the overall lowest amount of newly formed valves at 1 S_A and 15 S_A , while most new valves were produced at 30 S_A . Further, an increase of unstained valves from days 3 to 7 was observed for 15 S_A (Figure 8).

The PDMPO-staining approach confirmed the ecophysiological data well.

Effects of Temperature and Salinity on Diatom Lipid Droplets

The effects of temperature and salinity on the storage of lipids of the four diatom strains were determined calculating the lipid volume per cell on two separate days (Figure 9). *Melosira nummuloides* and *N. filiformis* stored their lipid droplets in several different shapes within considerably larger cells, while *Planothidium* sp. (st. 1) and *Planothidium* sp. (st. 2) exhibited two marginal lipid droplets within smaller cells (Figure 7). The effects of the different treatments within each strain were compared and tested for significance at day 7.

All strains exhibited rather uniform lipid volumes at day 3 during exponential growth for all temperature treatments. During the stationary phase at day 7, increases in lipid volume median over time were found for all tested strains at 17 and 22°C , ranging from 35 to 340%. At 7°C smaller or no changes occurred. Temperature effects were significant at day 7 for *M. nummuloides* and *Planothidium* sp. (st. 2) at 17°C , with the latter also being significantly changed at 22°C (Figure 9). *Planothidium* sp. (st. 1) did not show major lipid volume changes at the different temperatures. *Nitzschia filiformis* exhibited high variability, which led to mostly no statistical significance.

Salinity had contrasting and significant effects on the lipid volume of *M. nummuloides*, *N. filiformis* and *Planothidium* sp. (st. 1) (Figure 9). The lipid volume increased between days 3 and 7 in *M. nummuloides* and *Planothidium* sp. (st. 2) at all tested salinities from 8 to $204 \mu\text{m}^3$ per cell. *Planothidium* sp. (st. 1) showed minor differences between day 3 and day 7 with ambiguous results: a slight 20% decrease in lipid volume at 1 S_A and slight 40% increases at 15 and 30 S_A . In *N. filiformis* the lipid volume decreased for all three salinities over time. Significant changes in lipid volume after 7 days of treatment were found in all strains except *Planothidium* sp. (st. 2) but with different patterns as *N. filiformis* had the highest median lipid volume ($387 \mu\text{m}^3$ per cell) at the lowest salinity while *M. nummuloides* had its highest median ($204 \mu\text{m}^3$ per cell) at 15 S_A .

To determine the effects of temperature and salinity on the amount of lipid droplets per cell, lipid droplets were counted on both sampling days. *Planothidium* sp. always holds two lipid droplets. Therefore, only *M. nummuloides* and *N. filiformis* were included for in depth examination (Figure 10). The average amount of lipid droplets per cell for *M. nummuloides* was 16 and exhibited only slight changes from days 3 to 7 and no significant differences for all temperature and salinity treatments at day 7. *Nitzschia filiformis* showed relatively constant numbers of lipid droplets (3–8) between days 3 and 7 for the different temperatures but a 30% decrease for the lower two salinities, whereas at 30

TABLE 3 | P:R ratio of respective temperature dependent photosynthesis and respiration curves (**Figure 6**) of four benthic diatom strains [$n = 3$, except *Planothidium* sp. (st. 2) $n = 4$ and *M. nummuloides* $n = 2$] kept at 20°C in a f/2 Baltic Sea medium, 15 S_A.

Isolates	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
<i>Melosira nummuloides</i>	–	–	3.55 ± 2.64 ab	3.52 ± 3.17 a	2.47 ± 1.42 ab	1.21 ± 1.26 a	–	–
<i>Planothidium</i> sp. (st. 1)	–	1.39 ± 0.36 a	1.36 ± 0.46 a	1.29 ± 0.43 a	1.06 ± 0.82 ab	1.17 ± 0.22 a	1.20 ± 0.11 a	–
<i>Planothidium</i> sp. (st. 2)	–	5.16 ± 6.58 a	1.04 ± 0.43 a	0.73 ± 0.36 a	0.54 ± 0.18 a	0.41 ± 0.21 a	0.25 ± 0.16 b	–
<i>Nitzschia filiformis</i>	–	–	9.92 ± 4.67 b	3.61 ± 0.76 a	2.80 ± 0.67 b	1.78 ± 0.37 a	1.14 ± 0.17 a	0.67 ± 0.03

Different lowercase letters represent significant levels among all means as calculated per temperature by a one-way ANOVA (Tukey's test, $p < 0.05$); positive respiration and negative photosynthesis measurements were excluded from ratio calculations.

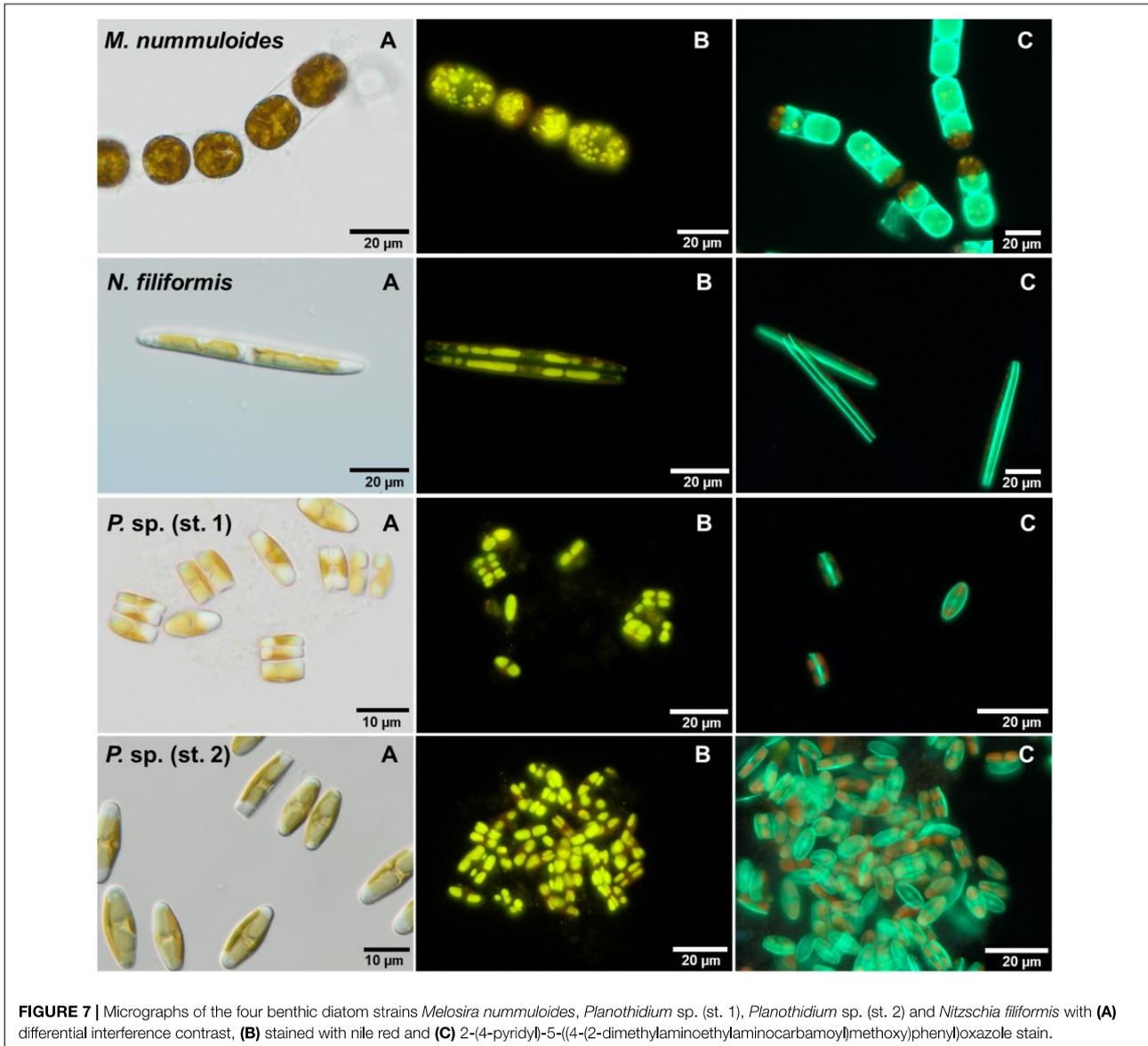


FIGURE 7 | Micrographs of the four benthic diatom strains *Melosira nummuloides*, *Planothidium* sp. (st. 1), *Planothidium* sp. (st. 2) and *Nitzschia filiformis* with (A) differential interference contrast, (B) stained with Nile red and (C) 2-(4-pyridyl)-5-((4-(2-dimethylaminoethyloaminocarbonyl)methoxy)phenyl)oxazole stain.

S_A the number of droplets was nearly equal. The comparison of the treatment effects of temperature and salinity, respectively, at day 7 revealed changes depending on the temperature but no significant effects of salinity.

DISCUSSION

The four investigated benthic diatom strains from the coastal Baltic Sea exhibited broad ecophysiological tolerances to the

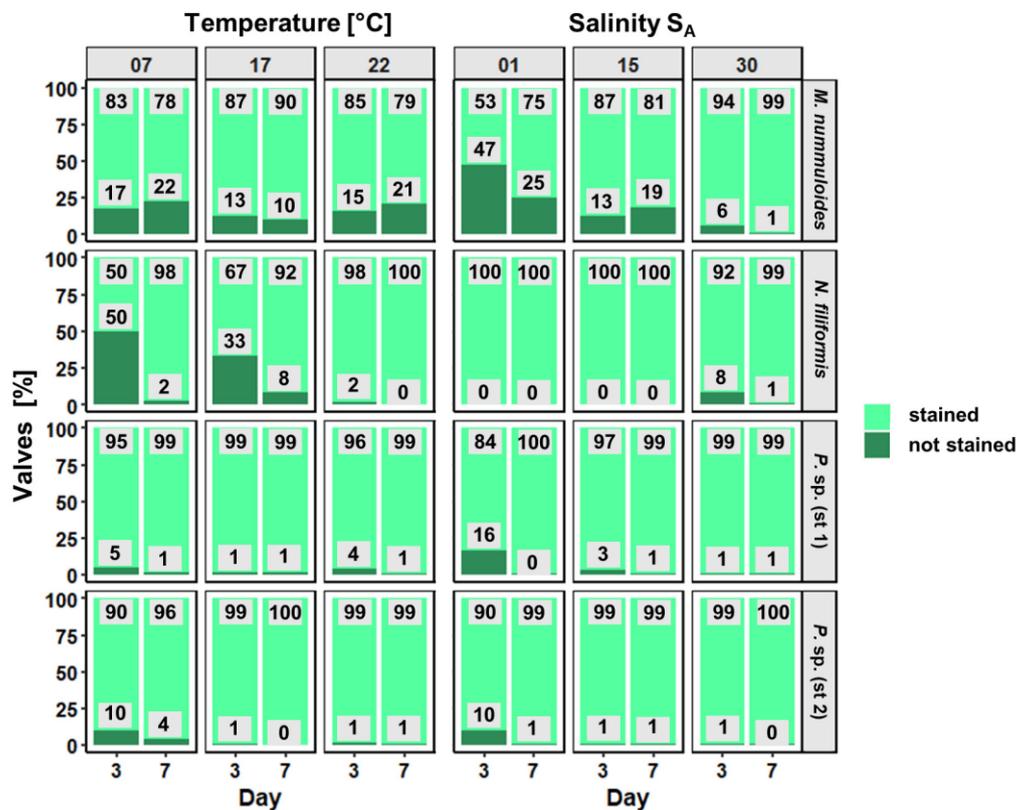


FIGURE 8 | Proportion (in %) of newly formed valves as function of temperature and salinity of four benthic diatom strains *Melosira nummuloides*, *Planothidium* sp. (st. 1), *Planothidium* sp. (st. 2), and *Nitzschia filiformis* for sampling days 3 and 7.

abiotic parameters light, temperature, and salinity, which in this study mimicked the annual fluctuations and extreme events at the study sites. The data presented support our assumption that these isolates are not only very well adapted to these stressors but are also able to withstand future abiotic changes that originate from expanding sea-land transition zones.

Growth

Salinity at the respective four study sites ranged between 9.2 and 13.6 S_A for the Baltic Sea and 1.2–8.9 S_A for the peatlands in 2019. In general, the four investigated benthic diatom strains exhibited growth rates ranging from 0.5 to 1.6 μ d⁻¹ at salinities from 1 S_A up to 39 S_A (except for *M. nummuloides*), which far exceeded the range of salinities occurring in their natural habitat by far. This is similar to the study of Woelfel et al. (2014), in which three benthic diatom species from a more westerly site of the Baltic Sea exhibited growth rates between 0.3 and 1.5 μ d⁻¹ at salinities ranging from 1 to 50 S_A. In accordance with our assumption, benthic diatom strains from the coastal peatlands are indeed euryhaline, growing well along salinity gradients between 1 and 39 S_A, and are therefore well adapted to recurring storm floods with saltwater intrusion into the freshwater habitat.

Although *M. nummuloides* is still considered euryhaline, it was not able to grow at 1 S_A. *Melosira nummuloides* is known as a

marine species (Crawford, 1973) leading to the assumption that it preferentially grows under higher salinities. This species was found on the sandy beach site of the Baltic Sea along the newly developed transition zone between the peatland Hütelmoor and the Baltic Sea after the latest storm surges, which gets flooded irregularly. This broad salinity tolerance of our *M. nummuloides* isolate in conjunction with intolerance of very low salinities confirms its marine characteristics.

Planothidium sp. (st. 1) exhibited its optimum growth rate at marine conditions, while salinity at the brackish location of origin normally only ranged between 2.5 and 5 S_A. Most of the *Planothidium* species are considered as freshwater species, however, there are a few known marine taxa (Algae Base). Therefore, the occurrence of *Planothidium* strains in the peatlands in combination with their salinity optimum may lead to the assumption that *Planothidium* sp. (st. 1) was swept into the peatland during the last saltwater inflow event in January 2019. This is in agreement with a recently submitted study on microphytobenthic primary production at an exposed sandy beach next to the Hütelmoor (Kuriyama et al., 2021), in which the authors report *Planothidium delicatulum* as most the abundant species (25% of the community) attached to sand grains. Even though salinity of the coastal German Baltic Sea in Mecklenburg-Pomerania rarely exceeds 14 S_A (Lippert et al., 2017) and

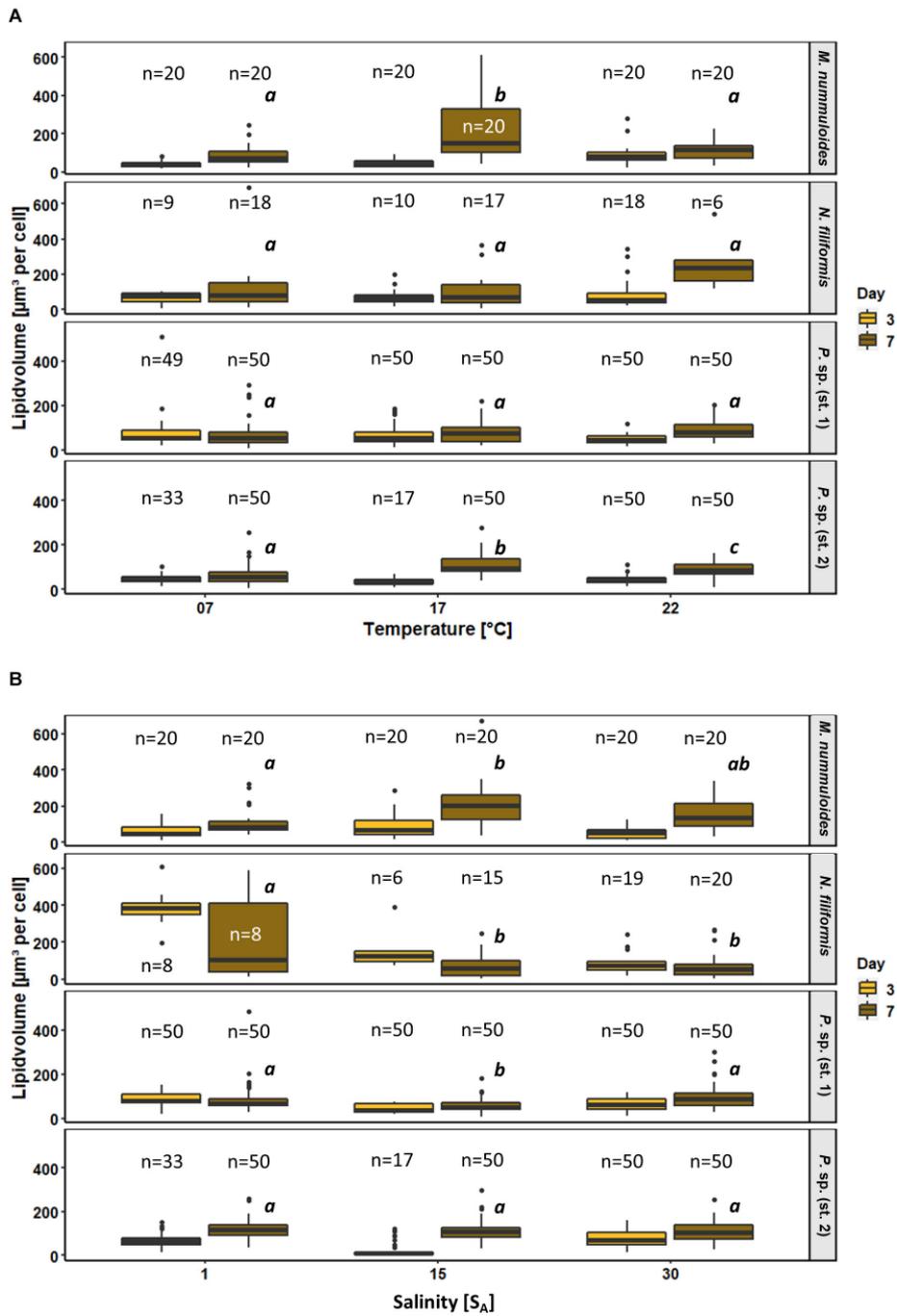
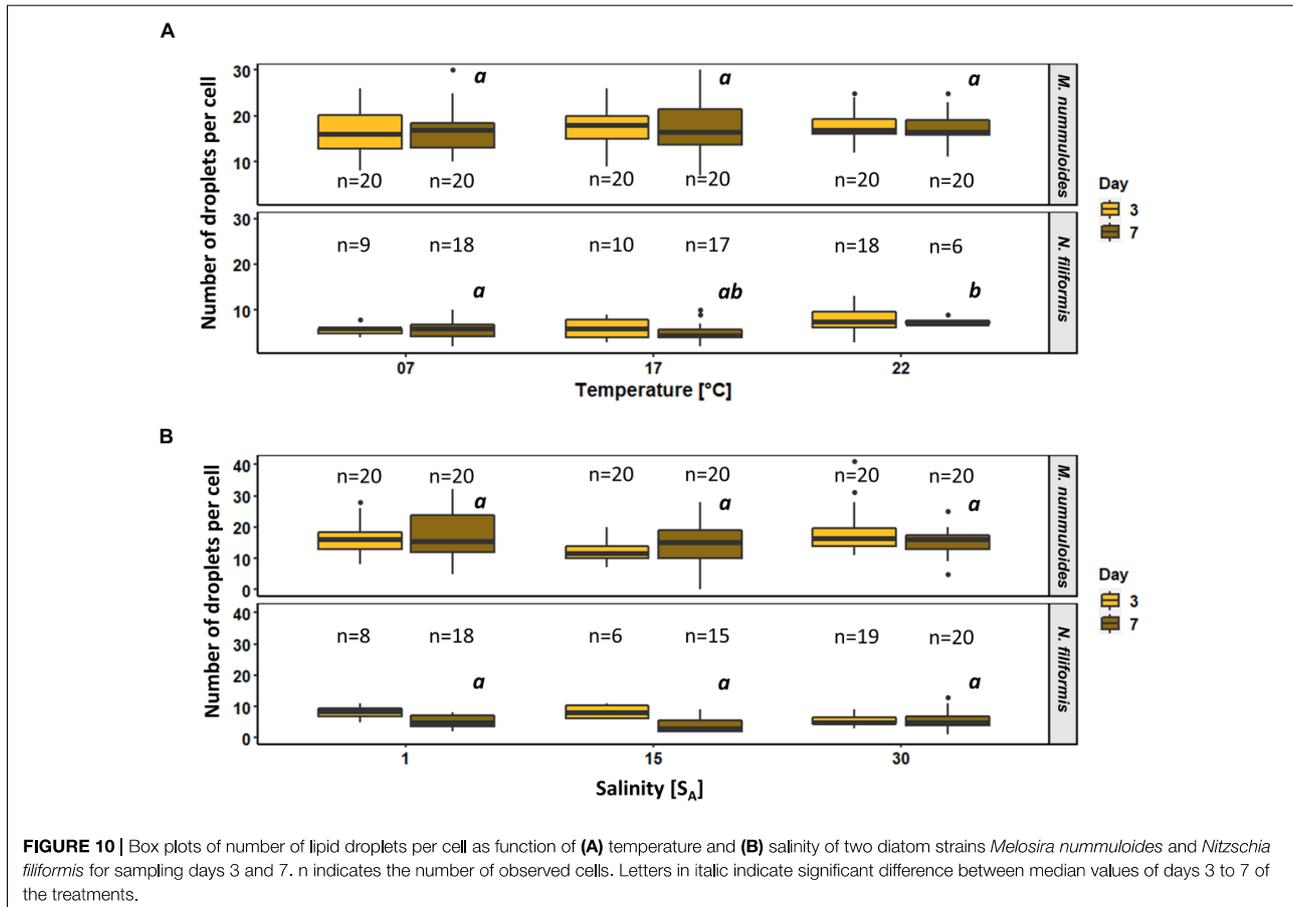


FIGURE 9 | Box plots of lipid volume as function of **(A)** temperature and **(B)** salinity of four benthic diatom strains *Melosira nummuloides*, *Planothidium* sp. (st. 1), *Planothidium* sp. (st. 2), and *Nitzschia filiformis* for sampling days 3 and 7. n indicates the number of observed cells. Letters in italic indicate significant difference between median values of days 3 to 7 of the treatments.

therefore is not considered a marine habitat, growth response of other marine species (Algae Base) found in the Baltic Sea such as *Navicula perminuta* (Woelfel et al., 2014), indicate that euryhaline marine species are also able to live in brackish environments.

In contrast to *Planothidium* sp. (st. 1), *Planothidium* sp. (st. 2), which was isolated from the peatland in Karrendorf with salinity ranging between 6 and 8 S_A, exhibited its maximum growth rate at 1 S_A. As there were no genetic differences in



the *rbcL* gene between both *Planothidium* strains, they can be considered as identical species. The conspicuous differences in the growth vs. salinity patterns, however, can be explained as development into two salinity ecotypes. The study site in Karrendorf has been regularly flooded for almost three decades allowing marine species that have been swept into this peatland to adapt to brackish/freshwater conditions. The concept of ecotypic differentiation is widely accepted in terms of physiological diversity and plasticity in seaweeds along the horizontal salinity gradient of the Baltic Sea (Nygard and Dring, 2008; and references therein), but also for microalgae such as *Skeletonema marinoi* from other regions (Saravanan and Godhe, 2010; and references therein). Ecotypes are variants of a species that are locally adapted to particular environmental conditions.

The brackish species *N. filiformis* (Algae Base) did not show significant differences in growth between 1 and 39 S_A . In contrast, Trobajo et al. (2011) found that an isolate of *N. filiformis* collected in the Ebro Estuary (Spain) was negatively influenced by salinities above 16 and 22 S_A with a slight decrease in growth, although precise growth rates were not provided. In addition, Trobajo et al. (2011) identified their isolate as *N. filiformis* var. *conferta* which probably is genetically different to our Baltic Sea strain.

The cell biological results on the formation of newly built diatom valves support the high growth rates at salinities between 1 and 30 S_A . Already at the first sampling after 3 days, almost

every valve was found as newly formed. Further, due to partly reaching the stationary phase within these days, in combination with clumping, less valves were formed after 7 days resulting in partly higher unstained valves than those that were found on day three. Therefore, a clear distinction of a significant influence of the treatment after 3 days of growth is impossible. These results were similar to valve formation in dependence of temperature. The temperature of the respective four study sites seasonally ranged from 4.0 to 21.9°C for the Baltic Sea and from 4.2 to 31.8°C for the peatlands (data from 2019). During sampling in June, the Baltic Sea temperature was 19.7°C and that of the Hütelmoor already 26.1°C. The maximum growth rate at 15–20°C of the four investigated strains was slightly higher than the average temperature in the respective habitat. In accordance with many other studies, cultivated diatoms tend to grow best at slightly higher temperatures compared to their natural habitat (Suzuki and Takahashi, 1995; Woelfel et al., 2014; Schlie and Karsten, 2017). For example, the occurrence of *N. filiformis* and *Melosira* spp. increased significantly at the discharge site of cooling water from a nuclear power plant which enhanced the water temperatures 6–10°C on average (Snoeijs, 2011).

The four benthic diatoms were able to grow well from 5 to 30°C with at least a 20% percentile of the growth rates, except for *N. filiformis*, which was not able to grow at 5°C. This coincides with the findings of Woelfel et al. (2014) on growth rates of

coastal Baltic Sea benthic diatoms, which ranged from 7 to 27°C with at least a 20% percentile of the growth rates. Admiraal (1977) studied 3 marine benthic diatom species from the Netherlands and reported a growth response between 4 and 25°C with the highest rates at 25°C. In a similar approach Scholz and Liebezeit (2012) investigated even 25 benthic diatom isolates from the German North Sea coast, and found optimum growth between 10 and 30°C, with strong species-specific reduction or inhibition at 4°C. The annual temperature of the Baltic Sea rarely surpasses 20°C. In contrast, adjacent peatlands exhibit ~30°C in summer, indicating that the origin of the respective diatom strains might be reflected in their growth rates, as the peatland diatoms are exposed to a much broader annual temperature range. However, no distinct differences in growth rates were found for the Baltic Sea and peatland diatom strains. This wide response can be explained as growth of diatoms is mainly impacted by physiological processes that are involved in photosynthesis, more specific in the uncoupling of C and N assimilation (Gleich et al., 2020; and references therein).

Temperature-Dependent Photosynthesis and Respiration

All four strains exhibited an efficiency of at least 20% over a large span of temperatures, clearly displaying eurythermal traits for photosynthesis and respiration. The strains isolated from the peatlands, *Planothidium* sp. (st. 1), *Planothidium* sp. (st. 2), and *N. filiformis*, displayed photosynthetic optima at higher temperatures ranging from 20 to 35°C whereas the Baltic Sea isolate *M. nummuloides* had its highest oxygen production at 10°C. This is according to the expectations that peatland species have to cope with higher temperature amplitudes. The small water bodies within the peatland contain large amounts of humic substances and hence absorb high solar radiation, resulting in higher water temperatures in summer compared to the Baltic Sea. Therefore, peatland algae must be adapted to higher temperature. However, there are still species-specific optima found, as *N. filiformis* was the only strain showing positive oxygen production over the whole range of temperatures. Prelle et al. (2019) used the same methodical approach to study the temperature dependent oxygen production in eight benthic diatom species from the southern Baltic Sea. Similar to the present study, they found that the optimum temperature for photosynthesis was lower compared to respiration. For diatoms, but also for green algae, temperature dependence of respiration and photosynthesis differs, as photosynthesis is more dependent on light, while respiration is mainly controlled by temperature dependent enzymatic activity (Atkin and Tjoelker, 2003; Karsten et al., 2016). This is partly confirmed in a more recent paper by Gleich et al. (2020), in which the authors indicate that light-dependent photosynthetic reactions are rather unaffected by temperature, while the carbon fixation reactions are not. In addition, a strong coupling of respiratory and photosynthetic activities is found in diatoms, which is explained by very tight physical interactions between chloroplasts and mitochondria (Bailleul et al., 2015). As a consequence, respiration is stimulated by light which results in

an optimum ATP/NADPH ratio for subsequent carbon fixation (Bailleul et al., 2015).

Light-Dependent Photosynthesis

Photosynthesis is primarily dependent on light availability. The PI-curves revealed species-specific photosynthetic activity with highest NPP_{max} for *N. filiformis* (106.94 μmol O₂ mg⁻¹ chl *a* h⁻¹) and lowest for *Melosira nummuloides* (28.02 μmol O₂ mg⁻¹ chl *a* h⁻¹). A previous study estimated a similar NPP_{max} of 23.12 μmol O₂ mg⁻¹ chl *a* h⁻¹ for a benthic *Melosira*-species from the Baltic Sea (Prelle et al., 2019). Additionally, lowest dark respiration rates as reported by Prelle et al. (2019) were similar to dark respiration rates of this study with *N. filiformis* exhibiting lowest rates.

For *N. filiformis* and *Planothidium* sp. (st. 2) a discrepancy in the absolute photosynthetic values at the same temperature between light-dependent and temperature-dependent photosynthesis was noticeable. While self-shading was avoided using always low cell numbers, the methodological approach of the temperature-dependent photosynthesis might explain such differences as the initial experimental temperature of 5°C strongly diverged from culture conditions (20°C). Therefore, a possible initial temperature shock is assumed, which lowered photosynthetic performance during measurements. While there was no temperature change during the light-dependent photosynthesis, strains exhibited higher photosynthetic activity.

Low light compensation points of 14.1–21.9 μmol photons m⁻² s⁻¹ in combination with low light saturation points of 29.8–72.6 μmol photons m⁻² s⁻¹ for all four isolates may indicate low light requirements for photosynthesis. The peatland diatoms are often exposed to high amounts of humic substances in their habitats, which lead to enhanced turbidity and hence shading in the small water bodies and therefore face low light availability. In contrast, the Baltic Sea is a highly dynamic environment concerning the light field. Besides meteorological conditions and seasonality, wind-induced waves and bioturbation can lead to temporal burial of benthic diatom cells, being faced with darkness inside the sediment. Vertical movement of raphid species can overcome such conditions. Moreover, *M. nummuloides* has been reported to inhabit organically polluted parts of the Clyde Estuary in Scotland, which can be deoxygenated with an increased content of suspended soil particles leading to high water turbidity. This isolate can live in depths exceeding the light penetration level at high tides (McLean et al., 1981) and therefore is able to tolerate low light climate similar to our peatland strains. While the low to moderate light availability in their natural habitat is reflected in low light requirements for photosynthesis in all four strains, it can also be a result of the low light conditions of 30–50 μmol photons m⁻² s⁻¹ during cultivation. Although Seródio and Lavaud (2011) showed that light requirements for photosynthesis are influenced by the laboratory growth conditions and hence might be the result of an acclimation to the culture maintenance, benthic diatoms are well known for their high photo-physiological plasticity (Ezequiel et al., 2015). Physiological and behavioral photoprotection (vertical movement into or out of the sediment) are the key mechanisms by which natural microphytobenthic

communities protect themselves against high incident solar radiation (Cartaxana et al., 2011). In addition, benthic diatoms can quickly adjust their photosynthetic apparatus to new light conditions (Glud et al., 2002), for example, by alterations of the size or composition of the photosynthetic units (Richardson et al., 1983). All PI-curves showed light saturation and three out of the four cultures exhibited—partly minor—photoinhibition at $1,500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. This is similar to the findings of Prellé et al. (2019) as only minor photoinhibition was reported in three out of eight benthic diatom species. However, a considerable decrease in photosynthetic activity was found in *M. nummuloides* above $1,300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. In diatoms, excessive light can be dissipated by non-photochemical quenching via the de-epoxidation state of the xanthophyll cycle (Serôdio et al., 2008). Further absorption of excessive light by the light harvesting complexes can lead to photoinhibition, the decrease of the photosynthetic efficiency, that cannot be safely dissipated as heat (non-photochemical quenching) and thereby lowering the quantum yield (Goss and Lepetit, 2015). Even though we cannot prove the underlying mechanism of the observed photoinhibition in the investigated species, only few explanations exist. One could be either due to damage of the photosynthetic apparatus (D1 protein of PSII), or because of a regulated and reversible process comprising the xanthophyll cycle (Goss and Lepetit, 2015) as response to $> 1,300 \text{ photons m}^{-2} \text{ s}^{-1}$, which still underline the high photosynthetic plasticity of benthic diatoms from the Baltic Sea coast.

Lipid Droplets

Under stress diatoms are known to store intracellular oil droplets through carbon fixation as reserve material during vegetative growth (D'Ippolito et al., 2015), however, the mechanism of high lipid production remains unclear (Sayanova et al., 2017). While the average lipid content of an oleaginous diatom is about 23% of the dry cell weight under habitual growth conditions, it can increase up to 45% dry cell weight under stress (Hu et al., 2008). In general, temperature effects on lipid volume were rather small in the four benthic diatom strains, as the first sampling did not exhibit significant difference in lipid droplet volume. Over time, the lipid droplet volume increased in most strains at 17 and 22°C due to a shift from exponential to stationary growth phase. Significant differences by the treatment in the stationary phase at day 7 were only found in *M. nummuloides* and *Planothidium* sp. (st. 2) at 17°C. Therefore, lower salinity represents unfavorable growth conditions for *M. nummuloides* that consequently should lead to a higher lipid accumulation at stationary phase. The number of lipid droplets per cell was not affected. The extent to which TAGs are produced is species- and strain-specific (Hu et al., 2008), as not every species is able to produce and accumulate TAGs in high amounts. Zhang et al. (2020) studied highly oleaginous diatom strains adapted to high temperatures. While the highest TAG content was obtained at 246.4 mg/g dry biomass (*Fistulifera* sp. HB236), the lowest TAG content was half as much (105.9 mg/g dry biomass; *Nitzschia palea* HB170). Diatoms from habitats with fluctuating environmental conditions have been shown to build up larger lipid storages to ensure their survival (Van Duong et al., 2015). Diatoms can adapt

to numerous stressors with broad tolerances toward salinity, temperature and light availability as previously mentioned. Therefore, it is likely that the temperature range in which lipid volume variations were studied was too narrow to induce a rapid build-up of large energy stores. Temperature effects on the lipid volume of the individual strains were investigated at temperatures (7, 17, and 22°C) at which all strains exhibited growth above 45–85% percentile of the optimum growth rates as well as positive oxygen production rates. Based on the growth rates and their wide temperature tolerance it may be assumed that the experimental conditions, under which temperature effects on lipid volume have been studied, did not lead to stress.

Analogous to temperature fluctuations, strong salinity fluctuations in the habitat may lead to the production and storage of biochemical energy reserves in microalgae in larger quantities than necessary under constant conditions (Lim et al., 2012; Van Duong et al., 2015). Results of *N. filiformis* suggest a salinity effect as lipid volume decreased significantly with increasing salinity. In contrast, the growth rates of this strain remained constant at salinities ranging from 1 to 39 S_A with no significant effect. Therefore, degradation of lipid storage as a stress response may prevent a decrease in growth at higher salinities. Interpretations have to be treated with caution, as these observations are limited due to the small number of evaluable cells. *Melosira nummuloides* did not grow at 1 S_A but grew well at salinities from 5 to 39 S_A, with no significant differences at increasing salinities. Therefore, lower salinity represents unfavorable growth conditions for *M. nummuloides* that consequently should lead to a higher lipid accumulation at stationary phase. However, our results cannot confirm this assumption. At the second sampling, lipid volume of *M. nummuloides* at 1 S_A exhibited the lowest volume compared to the higher salinities. A possible explanation is, that low salinities from the experimental and pre-culture phase constitute high stress levels over a certain period of time entailing that this strain was unable to build-up reserve lipids or even started to break down the lipid stores already. At the second sampling *Planothidium* sp. (st. 1) exhibited highest growth rates and an increase in lipid volume under marine conditions. Furthermore, an increase in lipid content for *Planothidium* sp. (st. 2) was observed under optimal growth conditions, which indicates the lipid production to a certain homeostasis also at non-stress, fast growth conditions. It is therefore questionable if lipid volume is a good indicator for stress responses in benthic diatoms and hence multiple experimental approaches are required for clear results.

CONCLUSION

In conclusion, all four benthic diatom strains exhibited euryhaline and eurythermal growth responses surpassing the annual fluctuations of the respective habitats. The optimum temperature for photosynthesis of the Baltic Sea diatom strain was at 10°C and those of the peatland diatoms at 20–35°C, reflecting the environmental temperature of the respective habitats. All strains exhibited low-light requirements, which is

of advantage in those habitats with high turbidity. The lipid content as a potential stress marker did not show clear results, indicating less suitability as previously thought. Overall, due to their eurythermal and euryhaline traits along with high photo-physiological plasticity, all four benthic diatom strains seem well adapted to cope and survive highly fluctuating abiotic parameters and gradients in the sea-land transition zone of the southern Baltic Sea coast.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

LP, MA, UK, and KG developed the idea and elaborated the concept. LP, MA, PD, TG, JJ, SM, LS, LV, and KG provided experimental and taxonomic data. All authors organized and conducted the data analyses, involved in writing the first draft of the manuscript, which was commented and edited by LP, MA, UK, and KG and finally accepted by all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2021.642811/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Table 1. Confidence intervals for temperature-dependent growth rates.

	2.5%	97.5%
<i>Nitzschia filiformis</i>		
maximal growth rate	0.749	0.970
optimum growth temperature	18.526	21.734
maximum growth temperature	30.537	34.189
maximal photosynthetic rate	47.374	60.930
optimum photosynthetic temperature	21.957	26.405
maximum photosynthetic temperature	40.000	45.459
maximum respirational rate	-34.759	-26.470
optimum respirational temperature	32.987	36.133
maximum respirational temperature	41.754	48.939
<i>Planothidium sp. (st. 1)</i>		
maximal growth rate	1.018	1.208
optimum growth temperature	18.706	20.973
maximum growth temperature	31.580	34.612
maximal photosynthetic rate	42.389	60.713
optimum photosynthetic temperature	26.013	30.383
maximum photosynthetic temperature	38.522	41.324
maximum respirational rate	-46.481	-37.961
optimum respirational temperature	28.073	41.036
maximum respirational temperature	40.967	77.819
<i>Planothidium sp. (st. 2)</i>		
maximal growth rate	1.074	1.198
optimum growth temperature	14.996	16.912
maximum growth temperature	32.460	35.205
maximal photosynthetic rate	18.639	26.084
optimum photosynthetic temperature	19.153	24.537
maximum photosynthetic temperature	35.307	37.968
maximum respirational rate	-52.748	-42.895
optimum respirational temperature	31.020	33.339
maximum respirational temperature	42.209	46.708
<i>Melosira nummuloides</i>		
maximal growth rate	1.058	1.209
optimum growth temperature	16.819	18.930
Maximum growth temperature	32.281	35.364
maximal photosynthetic rate	34.952	63.164
optimum photosynthetic temperature	0.341	17.808
maximum photosynthetic temperature	30.773	39.828
maximum respirational rate	-20.935	-13.124
optimum respirational temperature	29.478	40.193
maximum respirational temperature	35.731	62.395

4.3 Publication III

Photosynthesis, respiration, and growth of five benthic diatom strains as a function of intermixing processes of coastal peatlands with the Baltic Sea

Lara R. Prella and Ulf Karsten

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Article

Photosynthesis, Respiration, and Growth of Five Benthic Diatom Strains as a Function of Intermixing Processes of Coastal Peatlands with the Baltic Sea

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Abstract: In light of climate change, renaturation of peatlands has become increasingly important, due to their function as carbon sinks. Renaturation processes in the Baltic Sea include removal of coastal protection measures thereby facilitating exchange processes between peatland and Baltic Sea water masses with inhabiting aquatic organisms, which suddenly face new environmental conditions. In this study, two Baltic Sea and three peatland benthic diatom strains were investigated for their ecophysiological response patterns as a function of numerous growth media, light, and temperature conditions. Results clearly showed growth stimulation for all five diatom strains when cultivated in peatland water-based media, with growth dependency on salinity for the Baltic Sea diatom isolates. Nutrient availability in the peatland water resulted in higher growth rates, and growth was further stimulated by the carbon-rich peatland water probably facilitating heterotrophic growth in *Melosira nummuloides* and two *Planothidium* sp. isolates. Photosynthesis parameters for all five diatom strains indicated low light requirements with light saturated photosynthesis at $<70 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in combination with only minor photoinhibition as well as eurythermal traits with slightly higher temperature width for the peatland strains. Growth media composition did not affect photosynthetic rates.

Keywords: growth rate; flooding; ecocline; climate change; temperature; heterotrophy



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1. Introduction

The Baltic Sea in northern Europe is almost entirely enclosed by land with a catchment area of 1.74 million km² [1], and a coastline that is typically shaped by grassland, marches, peatlands, and inhabited areas. Depending on the meteorological conditions, only small exchange processes exist with the adjacent North Sea. In addition, considering the freshwater riverine discharge, this leads to a horizontal salinity gradient in the Baltic Sea ranging between $<3 S_A$ in the north and approximately $27 S_A$ in the south-east [2]. Being located in the temperate zone, abiotic factors such as temperature and light availability in and around the Baltic Sea exhibit strong seasonality.

In recent decades, the influence of climate change has become more concrete leading to new environmental conditions for the Baltic Sea. As a consequence of glacial melting and thermal expansion [3], the sea level of the Baltic Sea is continuously increasing by 2 mm yr⁻¹ in the southern Baltic Sea [4], which is enhanced by the glacio-isostatic subsidence of the southern Baltic Sea coastline [5]. Additionally, the enclosed characteristic of the Baltic Sea increases susceptibility to wind-induced sea level rise resulting in infrequent atmospheric pressure driven storm surges along the southern Baltic Sea coastline. In the past 60 years, the number of hours of storm surge with the entailing sea level rise of 0.5 m above sea level has increased significantly [6]. As a consequence, the adjacent terrestrial areas will be inundated by the Baltic Sea thereby facilitating hydrodynamic and biochemical exchange processes across the terrestrial-marine interface.

Nowadays, coastal terrestrial areas including farmlands and peatlands are often separated from the Baltic Sea by dunes and dikes as coastal protective measures. Due to this separation and the consequential artificial draining of the coastal areas, many peatlands have lost their original features and functions. Ecologically intact peatlands act as massive carbon sinks, that store up to twice as much carbon as all global forest biomass by covering only 3% of the world's terrestrial area [7]. Therefore, the number of coastal peatlands being restored, including an active or passive removal of the coastal protective structures, is increasing [8].

Along the German Baltic Sea coastline, restoration of various coastal peatlands is in progress, including a coastal fen within the nature reserve 'Heiligensee and Hütelmoor' in Mecklenburg Pomerania since the early 2000s [6]. The restoration plan includes removal of the near shore groyne systems as well as a gradual breakdown of the dune separating the Baltic Sea from this coastal peatland by natural forces, which resulted in a natural break in January 2019 due to an extremely strong storm surge (183 cm above mean sea level [9]). As a consequence, a massive Baltic Sea brackish water intrusion into the peatland took place. At the German Baltic Sea coast, additional peatlands are under restoration, such as the Drammendorfer Polder on the island Rügen, which was flooded by Baltic Sea water after a dike opening in November 2019 and a peatland in Karrendorf near Greifswald that has been under restoration for almost three decades [10,11]. These restoration projects strongly contribute to the storm surge-induced reinforced hydrodynamic and biogeochemical exchange processes across the sea-land transition zone, and also lead to the horizontal transportation of organisms and subsequently the intermixing of water bodies between peatlands and the coastal Baltic Sea.

Both ecosystems inhabit many microbial communities, including photosynthetic biofilms on top of soft bottom sediments, called microphytobenthos. The microphytobenthos is typically dominated by pennate diatoms that strongly contribute to the marine primary production [12,13]. Many pennate diatoms can vertically move in the sediment in response to environmental stressors [14,15] via the excretion of sticky extracellular polymeric substances (EPS) that stabilize soft bottom sediments [16]. Environmental stressors for these photosynthetic organisms mainly include light, temperature, and salinity gradients.

In both, coastal peatlands and the Baltic Sea, light availability seasonally and diurnally varies; however, both ecosystems also differ from each other in light conditions. Peatlands carry large amounts of dissolved and particulate organic matter resulting in high turbidity, which increase the incident light absorption [17]. This leads to a decrease in light penetration into the water column and hence low light conditions for benthic diatoms. In contrast, over the course of the seasons, the coastal shallow water of the Baltic Sea exhibits photon flux densities between 389 and 2117 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, which saturate benthic diatom photosynthesis [18]. However, prevailing strong hydrodynamic forces in the Baltic Sea such as winds and currents can lead to the burial of benthic diatom cells, with at least a temporal lack of light or even darkness. As a response to such unfavorable light conditions, many benthic diatoms are capable to vertically move along light gradients [19].

Coastal peatland and the adjacent Baltic Sea also exhibit differences in the annual temperature despite the proximity to each other. Along the shallow southern Baltic Sea, temperature seasonally varies between 4 °C in winter and 22 °C in summer [20]; while in the peatlands, temperature ranges from around the freezing point in winter to around 30 °C in summer. The large content of humic substances in small peatland water bodies results in high absorption of solar radiation and hence enhanced increasing water temperatures in summer compared to the Baltic Sea. These distinguished habitat temperature conditions are reflected in the benthic diatom temperature requirements for photosynthesis since peatland isolates have displayed optima between 20 to 35 °C, whereas those from the Baltic Sea have exhibited highest oxygen production at 10 to 20 °C [17,21,22]. The different photo-physiological response patterns in peatland and Baltic Sea benthic diatoms can be biochemically explained as carbon fixation is mainly controlled by temperature-dependent enzymes [23].

Photosynthesis and growth of benthic diatoms is also strongly dependent on nutrient supply. As a result of their location on top of the sediment, benthic diatoms take up nutrients from two sources, e.g., the water column and the nutrient-rich pore water within the sediment [24]. Therefore, benthic diatoms are strongly involved in biogeochemical exchange processes such as vertical nutrient fluxes across the sediment water interface [25]. Besides inorganic compounds, peatlands are also rich in particulate and dissolved organic carbon due to low oxygen-related degradation of organic material [26]. Unlike most photosynthetic organisms, some benthic diatom species are known for a heterotrophic and/or mixotrophic lifestyle using organic compounds to fuel their metabolism and maintain their photosynthetic ability when deprived from light [27].

With the now increasing sea level and storm surges, restored peatlands are expected to experience stronger and more abundant inundation events leading to increased exchange and intermixing processes of the water bodies of peatland freshwater and the brackish Baltic Sea. However, the impact of such drastic environmental changes on benthic diatom growth and photosynthesis has never been investigated, despite the tremendous importance of these organisms for their habitats.

Therefore, two Baltic Sea and three peatland benthic diatom strains were investigated for their ecophysiological response patterns of growth, photosynthesis, and respiration under simulated intermixing processes, which included treatment with varying growth media, light, and temperature conditions. Due to higher amounts of nutrients and organic compounds in the peatland, we expected higher growth rates in peatland-based media. In addition, we assumed eurythermal traits and a high photo-physiological plasticity in all benthic diatoms.

2. Materials and Methods

2.1. Study Site

Along the German Baltic Sea coast, four study sites were sampled in 2019 using undisturbed sediment cores and Petri dishes (diameter: 5 cm) for the isolation of benthic diatom strains originating from the shallow Baltic Sea and adjacent peatland (Figure 1). Samples from the coastal Baltic Sea were isolated along the Baltic shoreline in front of the nature reserve “Heiligensee und Hütelmoor” (54.22550 N, 12.17185 E). In this area, abiotic parameters such as wind, temperature, and salinity strongly fluctuate and thereby constantly shape the entire sampling site. Peatland samples were obtained from benthic of three coastal peatlands no deeper than 20–30 cm below water surface. The second sampling station (54.21212 N, 12.18343 E) was located in the peatland within “Heiligensee und Hütelmoor” which is underlying sporadic exchange processes with the Baltic Sea in relation to the sea level. The peatland Drammendorf (54.36971 N, 13.24384 E) was sampled prior to a dike removal in 2019; therefore, samples were not affected by Baltic Sea water influence. The third peatland sample was obtained from Karrendorf (54.15796 N, 13.38859 E); due to its proximity to the Greifswalder Bodden had been irregularly flooded with Baltic Sea water for the past three decades. Generally, temperature of the Baltic Sea shallow water in front of the “Heiligensee und Hütelmoor” annually varied between 4 °C in winter and 22 °C in summer with salinities ranging around 8.2 to 15.3 S_A [22]. The three peatland sites showed a similar annual temperature range between 4 °C up to 30 °C, with the sampling site in Karrendorf falling dry in summer. Salinity typically fluctuated in these peatlands between 0.5 and 5 S_A.

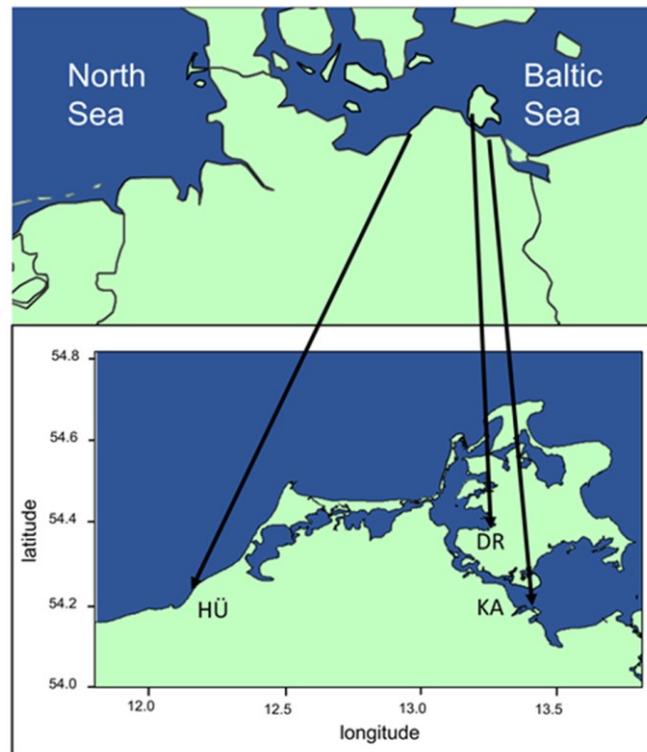


Figure 1. Map of northern Germany showing the three sampling sites HÜ (Hütelmoor), DR (Drammendorf) and KA (Karrendorf) next to the southern Baltic Sea.

2.2. Culture Establishment and Culture Conditions

For the experimental set up, the previously identified four diatom strains with the respective GenBank IDs from Prella et al. [22] were used: *Melosira nummuloides* (Melosiraceae, MW070612, strain PTM9a), *Planothidium* sp. (st. 1) (Achnanthesiaceae, MW070614, strain PTM25), *Planothidium* sp. (st. 2) (Achnanthesiaceae, MW070611, strain PTM7), and *Nitzschia filiformis* (Bacillariaceae, MW070613, strain PTM10). One additional strain originating from the Baltic Sea was used in the present study: *Hyalodiscus* cf. *scoticus* (Hyalodiscaceae, ON009273, strain PTM12). All strains were isolated according to Prella et al. [22]. In short, the upper 1 cm sediment layer of each sediment core was used for the establishment of clonal cultures. Sediment was incubated for approximately two weeks in Guillard's *f/2* medium [28,29] enriched with metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$; $10 \text{ g } 100 \text{ mL}^{-1}$) to a final concentration of 0.6 mM (further referred to as culture condition) using sterile filtered ($0.45 \text{ }\mu\text{m}$) Baltic Sea water (approximately 12 S_A) enriched with artificial sea salt (hw Marinemix® professional, hw Wiegandt Aquaristik, Krefeld, Germany) to a final salinity of 15 S_A (further mentioned as culture cultivation media) at $20 \text{ }^\circ\text{C}$ at $30\text{--}50 \text{ }\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ under a 16:8 h light:dark cycle (Osram Daylight Lumilux Cool White lamps L36W/840, Osram, Munich, Germany). Afterwards, single-cell isolation was performed using an inverted microscope (Olympus IX70, Olympus, Hamburg, Germany), until the establishment of unialgal cultures with low bacteria numbers ranging in maximum of 0.05–1% of the diatom volume (estimation via DAPI-staining). SEM (scanning electron microscopy) and light microscopy images were prepared following the same methodological approach as in Prella et al. (2021), and are deposited in the Supplemental Figures. Using molecular markers [22,30] and morphological identification with recent literature [31], diatom strains were determined. All cultures are available at the culture collection of the Department Applied Ecology and Phycology, University of Rostock. All

diatom strains were kept in culture cultivation media at 20 °C, 30–50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ under a 16:8 h light:dark cycle.

2.3. Specific Growth Rates

Specific growth rates of the five diatom strains in response to 10 different growth media were investigated using in vivo fluorimetry following the approach of Karsten et al. [32], Gustavs et al. [33], and Prella et al. [22]. This method uses chlorophyll, a fluorescence as a proxy for biomass and is particularly well suited for benthic diatoms.

The media were prepared with respect to possible flooding events between the Baltic Sea and adjacent peatland. These media were based on Baltic Sea water, peatland water, or a defined mixture of both (Table 1). All media were sterile filtered (0.45 μm) and stored at 5 °C until used. Salinity of the Baltic Sea water and the two saline peatland water growth media (GM9 and GM10) were adjusted to 15 S_A using artificial sea salt (hw Marinemix® professional, hw Wiegandt Aquaristik, Krefeld, Germany). As a control, f/2 and metasilicate were added to the media GM1, GM8, and GM10. Additionally, 219 μM NaNO_3 , (Carl Roth, Karlsruhe, Germany) as a source of inorganic nitrogen and 27 μM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (Carl Roth, Karlsruhe, Germany) as a source of inorganic phosphorus were added to the peatland media (GM7, GM8, GM9, and GM10; proportional to the mixed media GM4, GM5, and GM6) to mimic the highest nutrient values found in the sampling site of “Heiligensee und Hütelmoor”.

Table 1. Media composition for experimental growth medium GM1 to GM10 with the respective salinities (S_A) and respective nutrient additions of f/2 + metasilicate, inorganic nitrogen (N) as NaNO_3 and inorganic phosphorous (P) as $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ to supplement water basis.

Medium	Water Base	Salinity (S_A)	Nutrient Addition	pH
GM1	Baltic Sea	15	f/2 + metasilicate	7.6
GM2	Baltic Sea	15	-	7.4
GM3	Baltic Sea	15	219 μM N + 27 μM P	7.9
GM4	49/50 Baltic Sea + 1/50 peatland	15	219 μM N + 27 μM P (in the proportional peatland water)	7.6
GM5	9/10 Baltic Sea + 1/10 peatland	14.5	219 μM N + 27 μM P (in the proportional peatland water)	7.4
GM6	$\frac{1}{2}$ Baltic Sea + $\frac{1}{2}$ Peatland	8	219 μM N + 27 μM P (in the proportional peatland water)	7.2
GM7	Peatland	0.3	219 μM N + 27 μM P (in the proportional peatland water)	7.8
GM8	Peatland	0.3	f/2 + metasilicate	7.4
GM9	Peatland	15	219 μM N + 27 μM P (in the proportional peatland water)	7.1
GM10	Peatland	15	f/2 + metasilicate	7.3

All five diatom strains were cultivated in 15 mL of the respective medium ($n = 3$) in disposable Petri dishes with covered lids at 20 °C and 30–50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ under a 16:8 h light:dark cycle. Measurements proceeded every 24 h for 9 days by a MFMS fluorimeter (Hansatech Instruments, King’s Lynn, UK). Using blue LEDs (Nichia, Nürnberg, Germany) for the excitation of the chlorophyll *a* fluorescence; the resulting fluorescence was detected by an amplified photodiode and was separated from scattered excitation light through a long pass glass filter (RG 665; Schott, Mainz, Germany) and a bright-red gelatin filter (Lee, Brussels, Belgium) (see Karsten et al. [32] for further in-depth methodical details). The resulting relative fluorescence units were used for calculation of the specific growth rates (in μd^{-1}) during the logarithmic phase of the diatom strains applying the following equation: $N = N_t e^{\mu dt}$ (N —fluorescence intensity at the measuring day, N_t —initial

fluorescence intensity, dt —difference of time in days between measuring day and starting day, μ —growth rate) [33].

All five diatom strains were pre-incubated in the different media for four days before transfer to the experimental cultures to allow acclimation to the experimental condition.

2.4. Light Response Curves (PI-Curves)

The photosynthetic activity of the five diatom strains in response to increasing light availability and its dependency to the two selected cultivation media (GM6 and GM9) was measured in a so-called PI-Box following the approach of Prelle et al. [21]. These two media were chosen to investigate photosynthesis in likely occurring media changes after a storm surge events. The medium of GM2 was excluded due to a partially no growth response in the growth experiment. The PI-Box was constructed using water tempered oxygen electrode chambers (DW1, Hansatech Instruments, King's Lynn, UK) and air-tight cover lids with incorporated oxygen dipping probe DP sensors (optodes) (PreSens Precision Sensing GmbH, Regensburg, Germany). These probes were connected to a fiber optic oxygen transmitter via optical fibers (Oxy 4mini meter, PreSens Precision Sensing GmbH, Regensburg, Germany), measuring oxygen within the samples using the concept of oxygen fluorescence quenching. Samples consisted of 3 mL log-phase algal suspension ($n = 3$) and the addition of 30 μL of sodium bicarbonate (NaHCO_3 , final concentration 2 mM), to avoid carbon deficit during measurements. Each sample was exposed to 10 increasing light levels that were between 0 and $1377 \pm 44 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR) via LEDs (LUXEON Rebel1 LXML-PWN1-0100, neutral-white, Phillips, Amsterdam, Netherlands) at 20 °C. Starting with a 30 min respirational phase in darkness, light levels were increased every 10 min until reaching maximum oxygen production. Even biomass distribution within the samples for the entire duration of the measurement was ensured by magnetic stirrers set under the chambers. After oxygen measurements, chlorophyll *a* of each sample was determined for normalization towards oxygen measurements using 96% ethanol (*v/v*) for extraction. Chlorophyll *a* was determined spectrophotometrically at 750 nm and 665 nm (Shimadzu UV-2401 PC, Kyoto, Japan) [34].

All data were fitted using the photosynthetic model of Walsby [35], and the accompanying PI-parameters net primary production (NPP_{max}), respiration (R), light utilization coefficient (α), photoinhibition coefficient (β), light saturation point (I_k), and the light compensation point (I_c) were calculated.

2.5. Temperature Dependence of Photosynthesis and Respiration

The effect of increasing temperature between 5 and 40 °C in combination with two different cultivation media (GM6 and GM9) on photosynthetic and respiration rates of the five diatoms strains was determined following the approach of Karsten et al. [36] and Prelle et al. [21]. Using the same PI-Box as for the PI-curves, settings were adjusted to darkness during the respirational phase and photosynthesis saturated light conditions at $303.4 \pm 11 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for the photosynthetic phase. Again, 3 mL of log-phase algal suspension and the addition of 30 μL of sodium bicarbonate were added to the oxygen electrode chambers as they were tempered at 5 °C. Measurements started with a 20 min dark incubation phase before measurements for the 10 min respirational and 10 min photosynthetic phase. Temperature was then increased by 5 °C and each step was repeated until reaching 40 °C. Afterwards, chlorophyll *a* was determined as was conducted for the PI-curves. All data were fitted using the photosynthetic model of Yan and Hunt [37], excluding photosynthesis at 40 °C for *H. cf. scoticus*.

2.6. Statistical Analysis

Statistical analysis followed the same approach as Prelle et al. [22]. Calculations, including solver function by minimizing the sum of normalized squared deviations, fitting of the photosynthetic model of Walsby [35] and image creation, were performed using Microsoft office Excel (Version: 2016, Microsoft Corporation, Redmond, WA, USA) and R

(Version: 4.0.2, R Foundation for Statistical Computing, Vienna, Austria). Further, R was used for data fitting of the temperature-dependent photosynthesis using the model of Yan and Hunt [37], as well as calculation of significant levels using one-way ANOVA and the post-hoc Tukey's significant differences test (critical p -value < 0.05). Significant differences were indicated by lowercase and capital letters. In the Supplemental Table, confidence intervals of the modeled data are shown, which were calculated using library nls tools in R.

3. Results

3.1. Species Identification

Melosira nummuloides, *Planothidium* sp. (st. 1 and 2) and *Nitzschia filiformis* were previously identified by Prelle et al. [22]. The additional strain was morphologically identified as *Hyalodiscus* cf. *scoticus* using light microscopy and SEM images. Further results for the molecular analysis of the *rbcL* gene using NCBI Blastn [38] corresponded to 99.7% with *Hyalodiscus scoticus* s0284 (AB430660) and to 99.7% with *Hyalodiscus* sp. CCMP1679 (FJ002131).

3.2. Growth

Two Baltic Sea (Figure 2A,B) and three peatland (Figure 2C–E) benthic diatom strains were investigated for their specific growth rate as a function of 10 different growth media. Overall, the peatland water-based media (GM7–GM10) resulted in similar specific growth rates for *H. cf. scoticus* (Figure 2B) and *N. filiformis* (Figure 2D), and significantly ($p < 0.05$) higher specific growth rates for *M. nummuloides* (Figure 2A), *Planothidium* sp. (st. 2) (Figure 2C) and *Planothidium* sp. (st. 1) (Figure 2E) compared to the standard cultivation media (GM1). Specific growth rates in GM1 ranged between 0.31 d^{-1} (*H. cf. scoticus*) and 0.64 d^{-1} (*Planothidium* sp. (st. 1)) (Figure 2B,E). In the peatland species, only small differences were determined among the peatland water-based media, from GM7 to GM10, displaying the overall highest specific growth rates between 0.67 and 0.76 d^{-1} (*Planothidium* sp. (st. 1)), 0.60 and 0.75 d^{-1} (*Planothidium* sp. (st. 2)), and 0.51 and 0.61 d^{-1} (*N. filiformis*) (Figure 2C,D). The two Baltic Sea isolates *M. nummuloides* and *H. cf. scoticus* exhibited specific growth rates similar to those in the cultivation media for the saline peatland media (GM9 and GM10), between 0.56 and 0.67 d^{-1} and 0.27 and 0.38 d^{-1} , respectively (Figure 2A,B). A general trend for all five strains can be depicted for GM4, GM5, and GM6, as with the increasing proportion of peatland water-specific growth rates were stimulated by at least two times, although still slightly lower compared to GM7–GM10 (Figure 2). GM2 resulted in the overall lowest specific growth rates in all strains ranging between no growth and 0.12 d^{-1} , respectively. The addition of nutrients (NaNO_3 and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) to the Baltic Sea water (GM3) had a significant effect on specific growth rates of *M. nummuloides* and both *Planothidium* sp. strains compared to GM2 (Figure 2A,C,E). However, these rates were at least two thirds lower than highest overall specific growth rates under GM1, which also contained Na_2SiO_3 in addition to NaNO_3 and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$.

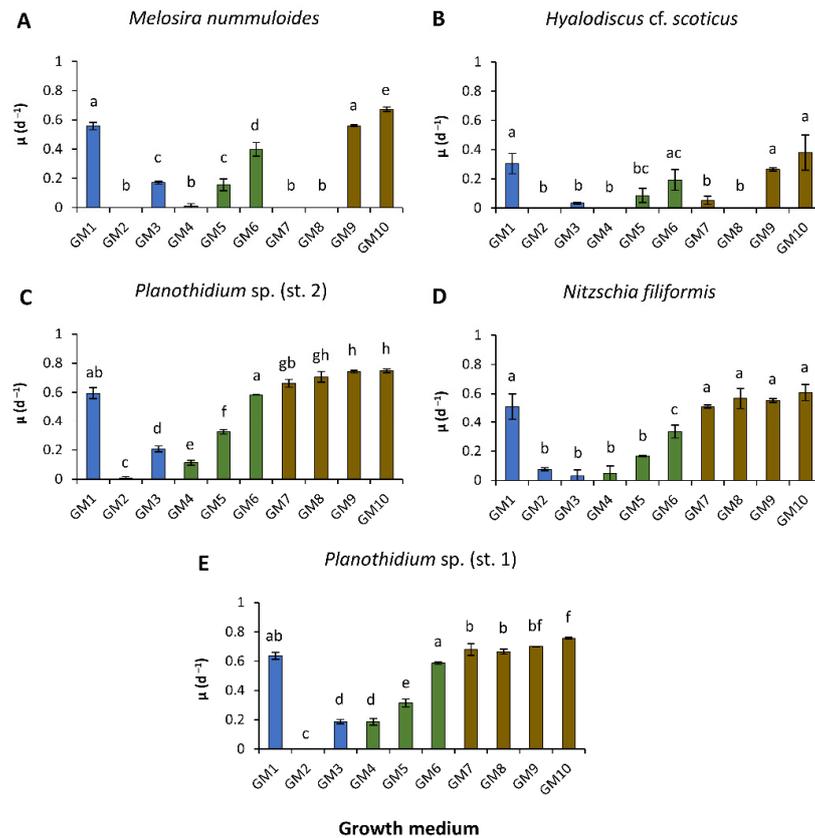


Figure 2. Specific growth rates (d^{-1}) in relation to 10 growth media (GM) (blue = Baltic Sea water base, brown = peatland water base, green = mixed water base) of two Baltic Sea (A,B) and three peatland benthic diatom (C–E) strains. Data represent mean values \pm standard deviation ($n = 3$). Different lowercase letters represent significant levels among all means as calculated by a one-way ANOVA (Tukey’s test, $p < 0.05$). (A) *Melosira nummuloides*, (B) *Hyalodiscus cf. scoticus*, (C) *Planothidium sp. (st. 2)*, (D) *Nitzschia filiformis*, and (E) *Planothidium sp. (st. 1)*.

3.3. PI-Curves

The light curves in combination with two growth media, GM6 and GM9, were investigated for both Baltic Sea (Figure 3A–D) and all peatland (Figure 4A–F) diatom strains, and the resulting parameters are shown in Table 2. The overall highest NPP_{max} was measured in *Planothidium sp. (st. 2)* with $79.6 \pm 19.5 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$ (GM9) and significant differences between treatments ($p < 0.05$, Table 2). Highest respiration was estimated with -79.5 ± 21 (*M. nummuloides*, GM6) and lowest with $-23.9 \pm 2.3 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$ (*Planothidium sp. (st. 1)*, GM9), and varied significantly ($p < 0.05$) between species (Table 2). However, no significant differences between media were determined. Light compensation points (I_c) ranged between $14.2 \pm 2 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (*Planothidium sp. (st. 1)*, GM9) and 46.2 ± 25.7 (*M. nummuloides*, GM6), while light saturation points (I_k) were higher between 19.6 ± 7.3 (*H. cf. scoticus*, GM6) and 68.0 ± 7 (*Planothidium sp. (st. 2)*, GM9) (Table 2). While photoinhibition (β) was found in half of the strains, but with relatively low values between -0.01 and $-0.02 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1} (\mu\text{mol photons m}^{-2} \text{ s}^{-1})^{-1}$, no photoinhibition was detected in the remaining samples (Table 2).

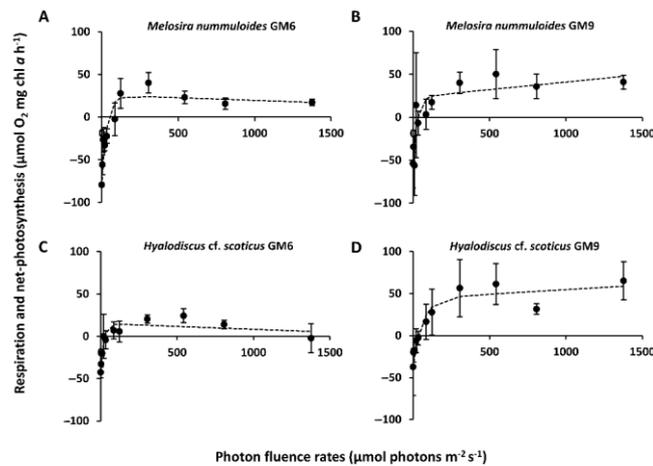


Figure 3. Photosynthesis and respiration rates ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$) of light curves ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) for two Baltic Sea benthic diatom strains kept at 20 °C in GM6 medium (A,C) and GM9 medium (B,D). Data represent mean values \pm standard deviation ($n = 3$, except *Melosira nummuloides* GM6 $n = 4$, *Hyalodiscus cf. scoticus* GM6 $n = 2$). (A) *Melosira nummuloides* GM6, (B) *Melosira nummuloides* GM9, (C) *Hyalodiscus cf. scoticus* GM6, and (D) *Hyalodiscus cf. scoticus* GM9. Data points were fitted by the model of Walsby [35].

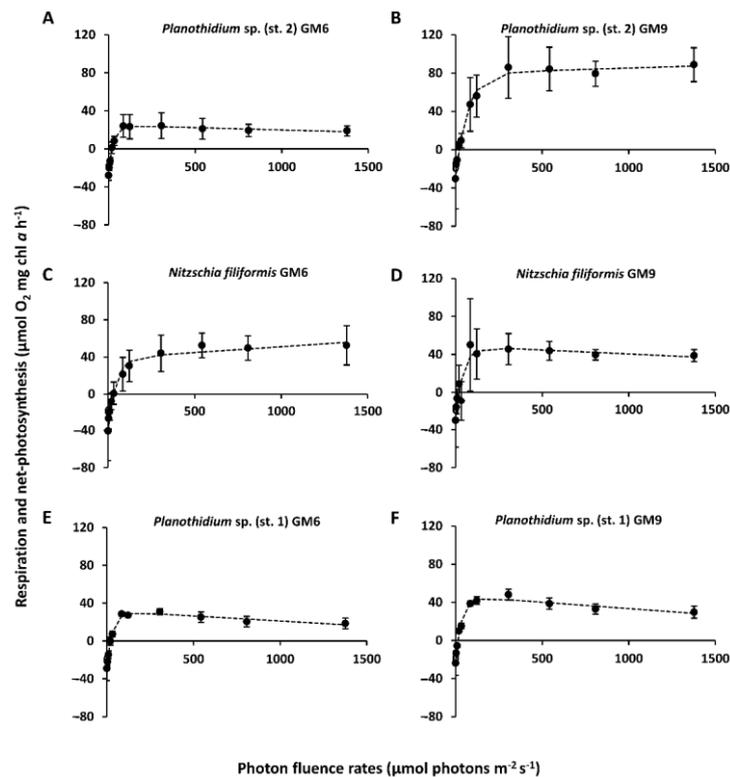


Figure 4. Photosynthesis and respiration rates ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$) of light curves ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) for three peatland benthic diatom strains kept at 20 °C in GM6 medium (A,C,E) and GM9 medium (B,D,F). Data represent mean values \pm standard deviation ($n = 3$, except *Planothidium sp. (st. 2)* GM6 and *Nitzschia filiformis* GM6 $n = 4$). (A) *Planothidium sp. (st. 2)* GM6, (B) *Planothidium sp. (st. 2)* GM9, (C) *Nitzschia filiformis* GM6, (D) *Nitzschia filiformis* GM9, (E) *Planothidium sp. (st. 1)* GM6, and (F) *Planothidium sp. (st. 1)* GM9. Data points were fitted by the model of Walsby [35].

Table 2. Parameter of respective PI-curves (Figure 3A–D and Figure 4A–F) for five benthic diatom strains ($n = 3$) kept at 20 °C in GM6 and GM9 media. Data represent mean values \pm standard deviation ($n = 3$, except *Melosira nummuloides* GM6 $n = 4$, *Hyalodiscus* cf. *scoticus* GM6 $n = 2$). Different lowercase letters represent significant levels among all means as calculated by a one-way ANOVA (Tukey’s test, $p < 0.05$). NPP_{max} represents the maximal oxygen production rate, α the initial slope of production in the light limited range, β the terminal slope of production in extensive light range (photoinhibition), I_k the light saturation point, and I_c the light compensation point.

Isolates	NPP_{max} ($\mu\text{mol O}_2 \text{ mg}^{-1}$ $\text{chl } a \text{ h}^{-1}$)	Respiration ($\mu\text{mol O}_2 \text{ mg}^{-1}$ $\text{chl } a \text{ h}^{-1}$)	α ($\mu\text{mol O}_2 \text{ mg}^{-1}$ $\text{chl } a \text{ h}^{-1}$) (μmol Photons m^{-2} s^{-1}) ⁻¹	β ($\mu\text{mol O}_2 \text{ mg}^{-1}$ $\text{chl } a \text{ h}^{-1}$) (μmol Photons m^{-2} s^{-1}) ⁻¹	I_k ($\mu\text{mol Photons}$ $\text{m}^{-2} \text{ s}^{-1}$)	I_c ($\mu\text{mol Photons}$ $\text{m}^{-2} \text{ s}^{-1}$)	NPP_{max} : Respiration
<i>Melosira nummuloides</i> GM6	23.31 \pm 14.55 ^a	-79.45 \pm 21.32 ^{cd}	3.23 \pm 2.57 ^a	-0.1 \pm 0.01 ^a	32.13 \pm 21.40 ^{ac}	46.19 \pm 25.69 ^a	0.31 \pm 0.12 ^d
<i>Melosira nummuloides</i> GM9	23.47 \pm 18.07 ^a	-53.90 \pm 4.81 ^{bd}	2.78 \pm 2.38 ^a	0.02 \pm 0.01 ^a	27.86 \pm 22.03 ^{abc}	32.58 \pm 19.60 ^a	0.44 \pm 0.32 ^{acd}
<i>Hyalodiscus</i> cf. <i>scoticus</i> GM6	14.12 \pm 11.22 ^a	-42.62 \pm 4.66 ^{ab}	2.89 \pm 1.26 ^a	-0.01 \pm 0.02 ^a	19.61 \pm 7.33 ^a	26.99 \pm 26.75 ^a	0.33 \pm 0.30 ^{cd}
<i>Hyalodiscus</i> cf. <i>scoticus</i> GM9	43.75 \pm 27.66 ^a	-37.29 \pm 5.83 ^{ab}	1.32 \pm 0.81 ^a	0.01 \pm 0.01 ^a	61.39 \pm 16.49 ^{abc}	37.28 \pm 22.63 ^a	1.17 \pm 0.54 ^{acd}
<i>Planothidium</i> sp. (st. 2) GM6	23.88 \pm 13.26 ^a	-27.89 \pm 4.70 ^{ab}	2.07 \pm 0.27 ^a	0.00 \pm 0.1 ^a	25.07 \pm 8.18 ^a	19.33 \pm 1.94 ^a	0.86 \pm 0.34 ^{acd}
<i>Planothidium</i> sp. (st. 2) GM9	79.58 \pm 19.51 ^b	-30.48 \pm 0.27 ^{ab}	1.62 \pm 0.47 ^a	0.01 \pm 0.00 ^a	68.03 \pm 7.06 ^{abc}	21.95 \pm 5.58 ^a	2.61 \pm 0.60 ^e
<i>Nitzschia filiformis</i> GM6	38.54 \pm 8.42 ^a	-40.68 \pm 11.32 ^{ab}	1.78 \pm 1.22 ^a	0.01 \pm 0.01 ^a	44.50 \pm 40.43 ^{abc}	31.61 \pm 26.22 ^a	0.95 \pm 0.22 ^{acd}
<i>Nitzschia filiformis</i> GM9	46.62 \pm 23.12 ^{ab}	-30.32 \pm 8.12 ^{ab}	1.89 \pm 1.12 ^a	-0.01 \pm 0.02 ^a	40.63 \pm 9.98 ^{abc}	20.33 \pm 13.04 ^a	1.54 \pm 0.51 ^{ab}
<i>Planothidium</i> sp. (st. 1) GM6	29.60 \pm 1.99 ^a	-28.95 \pm 2.79 ^{ab}	1.88 \pm 0.42 ^a	-0.01 \pm 0.00 ^a	31.07 \pm 7.68 ^{ac}	21.10 \pm 4.13 ^a	1.02 \pm 0.14 ^{acd}
<i>Planothidium</i> sp. (st. 1) GM9	44.01 \pm 3.90 ^a	-23.86 \pm 2.34 ^{ab}	2.07 \pm 0.73 ^a	-0.01 \pm 0.00 ^a	32.77 \pm 6.05 ^a	14.19 \pm 1.97 ^a	1.84 \pm 0.20 ^{abc}

3.4. Effect of Temperature on Photosynthesis and Respiration

The temperature increase from 5 °C to 40 °C in all benthic diatom strains led to a similar response pattern of the photosynthetic and respirational rates with significantly ($p < 0.05$) increasing signals until reaching an optimum followed by a strong decline (Figure 5A–D and Figure 6A–F). Except for *H. cf. scoticus*, both cultivation media compared to each other did not influence the photosynthesis/respiration rates under rising temperatures (Figure 5C,D). *Hyalodiscus* cf. *scoticus* exhibited a broader temperature tolerance for photosynthesis in GM9. Optimum temperature for photosynthesis of the Baltic Sea species was between 5 and 30 °C compared to 10 and 35 °C in the peatland species, with a similar pattern also observed for respiration (Figures 7 and 8). The photosynthetic rates of 20–80% of the maximum were between 5 and 35 °C for all three peatland strains (Figure 8); while in the Baltic Sea strains, only 0–19% of the maximum was reached at 35 °C (Figure 7). Generally, both cultivation media led to a shift in optimum ranges of the photosynthetic rates, with the most pronounced differences in *N. filiformis* and *H. cf. scoticus*. While treatment with GM9 led to a lower optimum temperature (10–20 °C) compared to GM6 (20–30 °C) in *N. filiformis*, *H. cf. scoticus* displayed a reverse pattern with lower optimum temperature for GM6 at 5 °C compared to GM6 at 25 °C (Figures 7A and 8A). A similar pattern could not be identified in the respirational rates of both strains (Figures 7A and 8A). Fitting of the data sets using the model of Yan and Hunt [37] also generally resulted in lower temperature requirements for optimum photosynthesis for the Baltic Sea strains compared to the peatland isolates, while temperature requirements for optimum respiration was rather similar (Tables 3 and 4). Treatment with both media resulted in higher maximum photosynthetic rates and lower respiration rates for all peatland strains and *H. cf. scoticus* in GM9 compared to GM6 (Tables 3 and 4); while reversely, GM6 stimulated higher photosynthetic and respirational rates in *M. nummuloides* (Table 3).

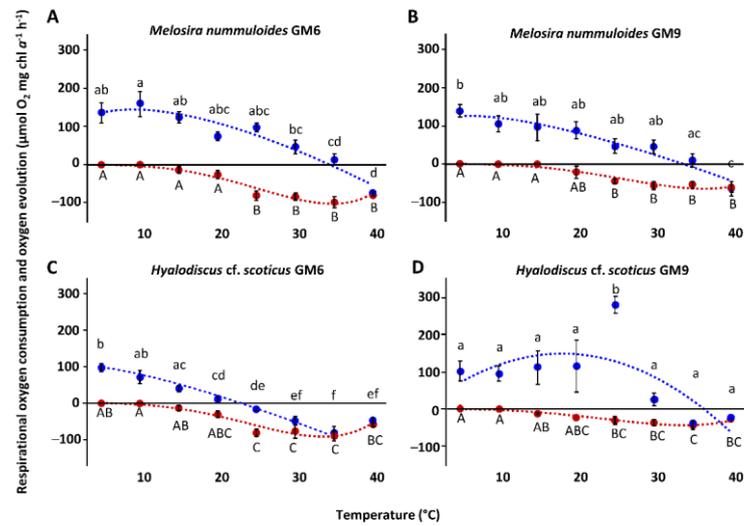


Figure 5. Photosynthetic (blue) oxygen production at $303.4 \pm 11 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and respiratory (red) oxygen consumption in darkness of two Baltic Sea benthic diatom strains as a function of increasing temperature. The measured data were fitted by the model of Yan and Hunt [37] (photosynthesis: blue dashed line (C), 40°C excluded); respiration: red dashed line). Cultures were kept in GM6 media (A,C) and GM9 media (B,D). Data represent mean values \pm standard deviation ($n = 3$). Different lowercase (photosynthesis) and capital letters (respiration) indicate significant means ($p < 0.05$, one-way ANOVA with post hoc Tukey's test). (A) *Melosira nummuloides* GM6, (B) *Melosira nummuloides* GM9, (C) *Hyalodiscus cf. scoticus* GM6, and (D) *Hyalodiscus cf. scoticus* GM9.

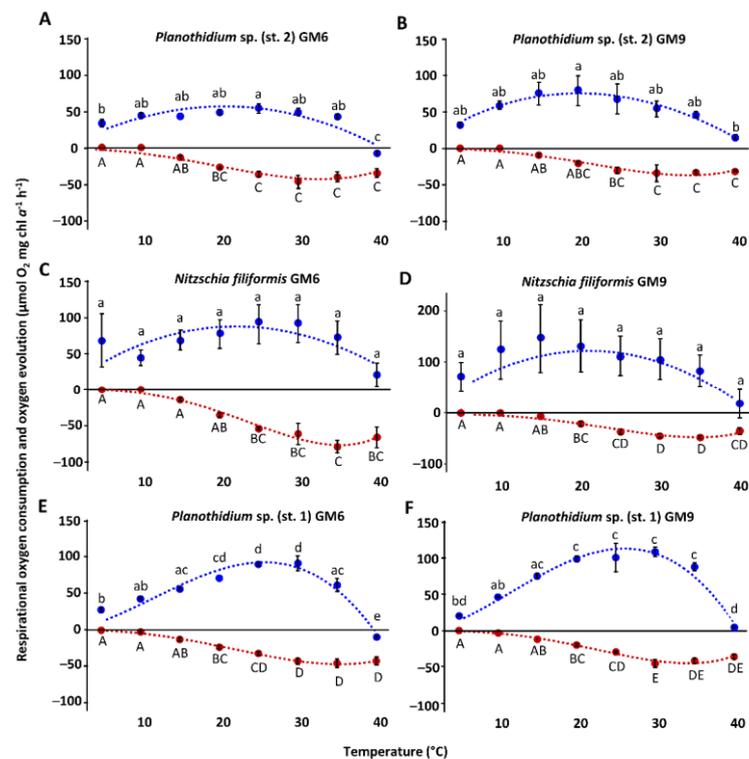
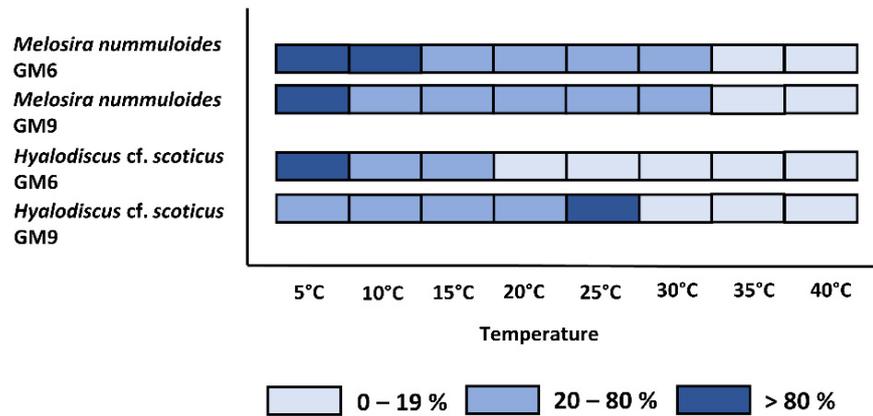


Figure 6. Photosynthetic (blue) oxygen production at $303.4 \pm 11 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and respiratory (red) oxygen consumption in darkness of three peatland benthic diatom strains as a function of

increasing temperature. The measured data were fitted by the model of Yan and Hunt [37] (photosynthesis: blue dashed line; respiration: red dashed line). Cultures were kept in GM6 media (A,C,E) and GM9 media (B,D,F). Data represent mean values \pm standard deviation ($n = 3$). Different lowercase (photosynthesis) and capital letters (respiration) indicate significant means ($p < 0.05$, one-way ANOVA with post hoc Tukey's test). (A) *Planothidium* sp. (st. 2) GM6, (B) *Planothidium* sp. (st. 2) GM9, (C) *Nitzschia filiformis* GM6, (D) *Nitzschia filiformis* GM9, (E) *Planothidium* sp. (st. 1) GM6, and (F) *Planothidium* sp. (st. 1) GM9.

A) Photosynthesis



B) Respiration

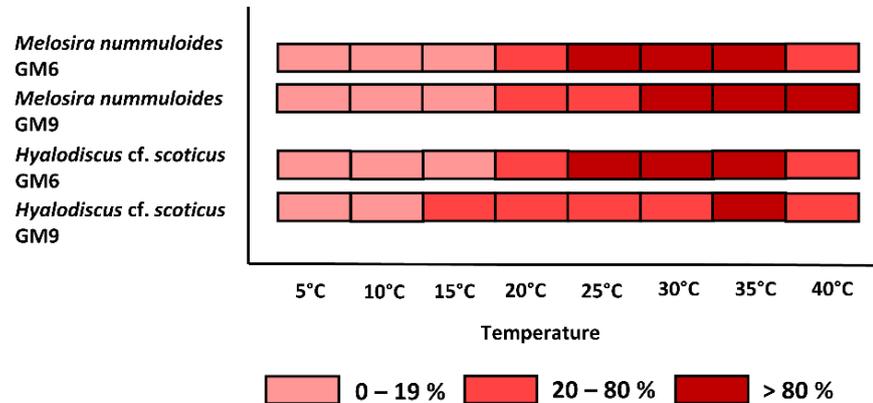
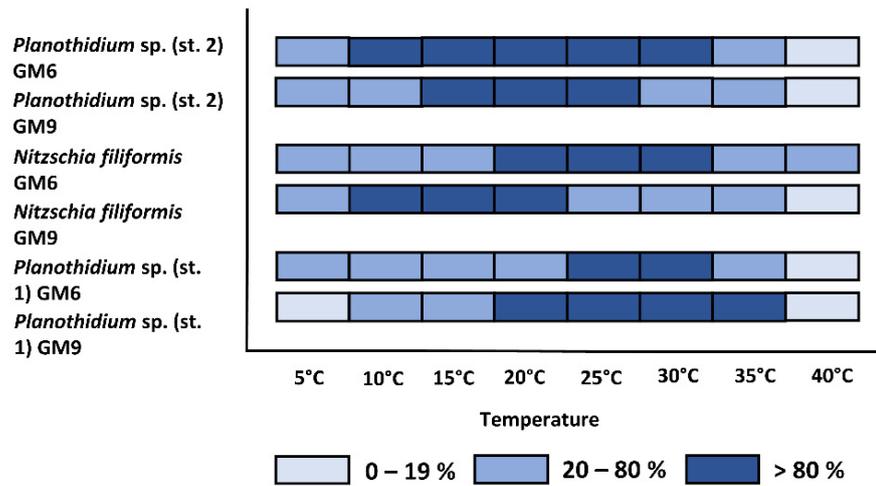


Figure 7. Effect of temperature on (A) photosynthetic oxygen evolution and (B) respirational oxygen consumption of two Baltic Sea benthic diatom strains in two different cultivation media (GM6 and GM9). Dark blue/red symbols represent highest photosynthesis at >80% percentile, medium blue/red symbols within 20 and 80% percentile and light blue/red symbols <20% percentile. Data represent mean values ($n = 3$).

A) Photosynthesis



B) Respiration

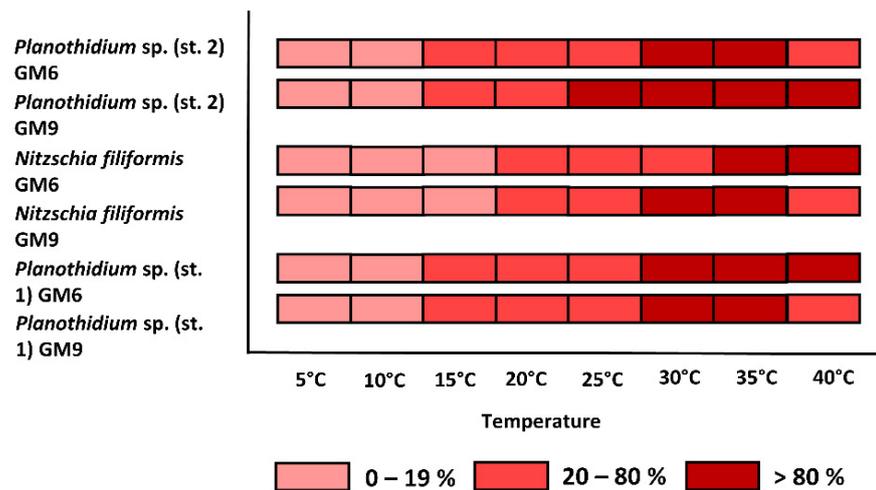


Figure 8. Effect of temperature on (A) photosynthetic oxygen evolution and (B) respirational oxygen consumption of three peatland benthic diatom strains in two different cultivation media (GM6 and GM9). Dark blue/red symbols represent highest photosynthesis at >80% percentile, medium blue/red symbols within 20 and 80% percentile and light blue/red symbols <20% percentile. Data represent mean values (n = 3).

Table 3. Results of model calculation for temperature-dependent photosynthetic and respirational rate ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$) of two Baltic Sea benthic diatom strains in the growth media GM6 and GM9 (Table 1) following the model of Yan and Hunt [37].

		<i>Melosira nummuloides</i> GM6	<i>Melosira nummuloides</i> GM9	<i>Hyalodiscus cf. scoticus</i> GM6	<i>Hyalodiscus cf. scoticus</i> GM9
Photosynthesis	maximal photosynthetic rate	144.5	125.9	74.1	148.8
	optimum temperature (°C)	9.0	6.3	4.2	18.2
	maximum temperature (°C)	34.4	33.8	22.5	36.4
	residual sum-square	24,794	28,054	7083	158,658
	temperature (°C) range for	optimal photosynthesis (80% photosynthetic rate)	2.5–18.8	1.1–16.1	0.7–10.6
photosynthesis (20% photosynthetic rate)		0.0–31.3	0.0–29.9	0.0–19.9	1.9–34.5
Respiration	maximal respirational rate	−100.9	−64.5	−90.6	−42.5
	optimum temperature (°C)	34.6	36.6	33.3	33.7
	maximum temperature (°C)	44.6	47.7	43.3	45.0
	residual sum-square	5415	4764	7797	1704
	Temperature (°C) range for	optimal respiration (80% respirational rate)	28.1–39.6	29.4–42.1	26.8–38.3
respiration (20% respirational rate)		16.4–45.8	16.4–46.7	15.0–42.4	14.1–43.9

Table 4. Results of model calculation for temperature-dependent photosynthetic and respirational rate ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$) of three peatland benthic diatom strains in the growth media GM6 and GM9 (Table 1) following the model of Yan and Hunt [37].

		<i>Planothidium sp. (st. 2)</i> GM6	<i>Planothidium sp. (st. 2)</i> GM9	<i>Nitzschia filiformis</i> GM6	<i>Nitzschia filiformis</i> GM9	<i>Planothidium sp. (st. 1)</i> GM6	<i>Planothidium sp. (st. 1)</i> GM9
Photosynthesis	maximal photosynthetic rate	57.1	76.1	87.9	138.6	93.1	113.3
	optimum temperature (°C)	20.6	20.1	22.1	18.4	25.7	26.1
	maximum temperature (°C)	40.7	42.4	45.3	42.2	39.7	40.7
	residual sum-square	2699	7837	30,816	95,423	2833	4240
	temperature (°C) range for	optimal photosynthesis (80% photosynthetic rate)	11.5–29.6	10.6–29.9	11.9–32.4	8.9–28.7	17.9–32.4
photosynthesis (20% photosynthetic rate)		2.3–38.6	1.7–40.0	2.1–42.9	1.1–39.5	6.7–38.4	6.6–39.3

Table 4. Cont.

		<i>Planothidium</i> sp. (st. 2) GM6	<i>Planothidium</i> sp. (st. 2) GM9	<i>Nitzschia</i> <i>filiformis</i> GM6	<i>Nitzschia</i> <i>filiformis</i> GM9	<i>Planothidium</i> sp. (st. 1) GM6	<i>Planothidium</i> sp. (st. 1) GM9
Respiration	maximal respirational rate	−45.5	−35.9	−75.7	−49.8	−47.1	−44.4
	optimum temperature (°C)	33.0	34.1	35.4	33.8	35.4	34.2
	maximum temperature (°C)	45.4	47.1	45.4	44.6	49.1	45.5
	residual sum-square	1143	1062	3299	670	610	429
temperature (°C) range for	optimal respiration (80% respirational rate)	25.3–39.1	26.1–40.4	28.8–40.4	26.9–39.1	27.0–42.1	26.9–39.8
	respiration (20% respirational rate)	12.5–44.3	12.7–45.9	14.6–43.4	14.6–43.6	132.–47.8	14.3–44.5

4. Discussion

As already mentioned in the introduction, with the increasing sea level rise and strength of storm surges along the southern Baltic Sea, coastal peatlands will be increasingly more affected by brackish water inundation and the resulting biota transported in the water masses, with so far unstudied ecological consequences for such wetlands. Therefore, we simulated different habitat conditions and evaluated the effects on ecophysiological traits of benthic diatoms as important primary producers, which originated from the shallow Baltic Sea and adjacent coastal peatlands.

4.1. Growth

The flooding of coastal peatlands by Baltic Sea water results in the establishment of different physico-chemical conditions for the inhabiting of aquatic organisms. With respect to such processes, 10 different experimental growth media consisting of different proportions of Baltic Sea and peatland water and different nutrient conditions resulted in species-specific responses with significantly higher specific growth rates of up to 0.8 d^{-1} in the peatland-based media. From an ecological perspective, growth is the physiological key indicator of the organism's fitness in response to its environment, which is controlled by multiple abiotic factors such as light conditions and temperature in benthic diatoms [39–41]. Growth is also driven by the availability of inorganic nutrients to fuel the photosynthetic metabolism [42]. Typically, coastal fens carry large amounts of the essential macronutrients nitrogen and phosphorus, due to low oxygen levels and the resulting low redox potentials. Therefore, benthic diatoms benefit from the high nutrient availability in the peatland-based media as reflected in the high specific growth rates of both peatland and Baltic Sea originating strains.

The two Baltic Sea strains *Melosira nummuloides* and *Hyalodiscus* cf. *scoticus* did not grow in freshwater-based peatland media compared to the saline peatland media, pointing to some salt requirements. Salinity is known to influence growth on a species-specific basis [43,44], and *M. nummuloides*, for example, is described from marine habitats [45]. Corresponding to the present study, Prella et al. [22] reported a euryhaline growth response for *M. nummuloides* from 5 to 39 S_A , with inhibited growth at 1 S_A . In addition, *Planothidium* sp. (st. 2), *Nitzschia filiformis* and *Planothidium* sp. (st. 1) exhibited euryhaline growth responses between 1 and 39 S_A [22], supporting the present findings of growth in all tested peatland water-based media.

The intermixing process between the Baltic Sea and coastal peatlands would be a rather transitional process, resulting in different proportions of peatland to Baltic Sea water masses. Under such conditions, all five benthic diatom strains, regardless of their originating habitat, were in principle able to exhibit growth, reflecting at least an initial growth phase after a storm surge-induced intermixing of water masses from both habitats. However, the limited

lower salinity tolerance of the Baltic Sea strains will not allow permanent occurrence in the peatland with further re-freshening of the wetland.

On the other hand, peatland diatoms transferred to the Baltic Sea medium did not grow, and the same was true for the Baltic Sea strains, which can be explained by nutrient deficiency. Measurements for nitrogen and phosphorus in the Baltic Sea medium compared to the modified peatland water-based media revealed much lower concentrations, which although reflecting the monthly nutrient measurements for the shallow Baltic Sea water column ranging between 1.2 and 11.2 $\mu\text{mol/l}$ for nitrogen (as NO_3^- , NO_2^- and NH_4^+ combined) and 0.1 to 1.2 $\mu\text{mol/L}$ for dissolved and hence bioavailable orthophosphate (Prelle, unpublished data), they do not reflect sediment pore water conditions. Low availability of nitrogen and phosphorus can lead to nutrient deficiency in microalgae, thereby lowering specific growth rates [46]. Under natural conditions, benthic diatoms typically benefit from the nutrient-rich pore water [24]. Nevertheless, treatment of the nitrogen and phosphorous enriched Baltic Sea medium (GW3) did not lead to enhanced specific growth rates as found for the peatland media, indicating other compounds (e.g., trace metals, vitamins), which might be essential for diatom growth.

Compared to other phototrophic organisms, silicate is one of the key nutrients for diatom growth, as it is used for the biosynthesis of the characteristic silicate frustule [47]. In contrast to the peatland water, the Baltic Sea showed relatively low silicate concentrations (Baltic Sea $< 1.0 \text{ SiO}_2 \text{ mg L}^{-1}$, peatland $> 6.0 \text{ SiO}_2 \text{ mg L}^{-1}$; using the SiO_2 visual aquarium quick assay (JBL, Neuhofen Germany)). Therefore, experimental strains may have suffered silicate deficiency as reflected in low or declined growth. Recent studies conducted in the Baltic Sea in front of the Hütelmoor reported submarine ground water discharge from the peatland into the Baltic Sea [48]. Further, nutrient fluxes between the pore water and the overlying water column were increased up to four times under typical hydrodynamic conditions found in this area. In addition, pore water was analyzed using oxygen isotopes proving a significant source of nitrogen, phosphorus, and silicate [6]. Therefore, benthic diatoms should be supplied with sufficient silicate from the pore water when inhabiting the sediment surface.

As already mentioned, growth was additionally stimulated by the peatland-derived medium. Due to low decomposition rates as a result of low oxygen levels, peatland waters carrying high amounts of dissolved organic carbon and across the sediment–water interface of the Baltic Sea in front of the Hütelmoor, around 250 mg/L were measured [6] compared to 1.1–4.8 mg/L in the water column at different sites [49]. The available organic carbon could have fueled a heterotrophic and/or mixotrophic metabolism in the diatoms. Both facultative and obligate heterotrophic pathways have been reported in diatoms [27] using organic compounds such as glucose via glycolysis to fuel their metabolism [50]. While the chemical composition of organic compounds in the utilized peatland water is unclear, the study by Prelle et al. [21] indicated heterotrophic growth in the Baltic Sea diatom *Actinocyclus octonarius* incubated in Hütelmoor peatland water. So far, detailed studies on heterotrophic or mixotrophic growth in the Baltic Sea and peatland benthic diatoms are missing.

Intermixing of water masses from both habitats may also lead to changes in pH, as the Baltic Sea exhibits higher pH values at 8.3 than the peatland at 6.8 (Prelle and Mutinova, unpublished data). At least the planktonic diatom *Skeletonema costatum* was not affected in growth by pH of 6.5 and 8.5 [51], and we assume similar response for benthic diatoms.

4.2. Light

The strong turbidity of peatlands results in lower light conditions in the same depths compared to the Baltic Sea, pointing to benthic diatoms in wetlands with lower light requirements than those in the Baltic Sea. Calculated PI-curve parameters confirm this assumption, as maximum photosynthetic rate was already reached between 25 and 68 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in combination with low light compensation points ranging between 19 and 32 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the three peatland diatom strains. The Baltic Sea benthic diatom strains exhibited light saturated photosynthesis between 20 and 61 μmol

photons $\text{m}^{-2} \text{s}^{-1}$ but slightly higher light compensation points of 27–46 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ indicating low light acclimation. Despite stronger light penetration in the Baltic Sea compared to the peatland, the shallow coastal Baltic Sea is a dynamic wind-driven environment leading to strong sediment resuspension, thereby causing regularly low light conditions due to turbidity or even cell burial. On the other hand, transportation into shallower depths within both habitats can lead to the exposure to higher photon fluence rates. All five diatom strains exhibited unaffected photosynthesis over wide photon fluence rates with only minor photoinhibition at up to 1400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, indicating a high photo-physiological tolerance and plasticity. In case of unfavorable high or low light conditions, diatoms reveal an array of acclimation mechanisms. On top of the sediment, pennate diatoms avoid high light exposure by vertically moving down into the sediment via the excretion of EPS, such as found in *N. filiformis* [14,15,52]. Diatoms have additional biochemical abilities to avoid oxidative damage of the photosystem II via the dissipation of excess energy as heat resulting from overexposure to strong light conditions [53]. The process of non-photochemical quenching of chlorophyll *a* fluorescence is induced within a few seconds to minutes [53]. Further, an accumulation of the photoprotective xanthophyll pigment diatoxanthin via the reaction of diadinoxanthin de-epoxidation weakens the oxidative stress under high light conditions [54]. However, other mechanisms such as the antioxidative potential (antioxidants and antioxidative enzymes) [55] must be considered for light acclimation. Photosynthetic activity of the five diatom strains was not affected by the different media.

4.3. Temperature

Contrary to the very dynamic coastal Baltic Sea, the adjacent peatlands are characterized as shallow, still, and often dark habitats with a low albedo. In summer, these traits lead to a rapid warming of the respective water bodies reaching over 30 °C, while in winter temperatures around the freezing point and ice coverage can be observed (personal observation).

Generally, all five diatoms exhibited broad temperature tolerances with moderate photosynthesis from 5 to 35 °C. Despite the differences in annual temperature ranges of the respective habitats and species-specific responses, rather similar photosynthetic rates were found in the peatland and Baltic Sea diatoms, independent of the media tested. In accordance with other studies, the optimum temperature for photosynthesis is generally found to be lower compared to the respiration [21,56,57]. The obvious decoupling of both processes may be explained by the photosynthetic light reactions mainly being light driven, and even indicated to be rather unaffected by temperature [58] in comparison to respiration, which is mainly controlled by temperature-dependent enzymes [59,60]. Nevertheless, in the photosynthetic dark reaction the carbon fixing enzyme RuBisCO (Ribulose-1,5-bisphosphat carboxylase-oxygenase) is losing its specificity towards CO₂ with increasing temperature [61].

5. Conclusions

In conclusion, all five benthic diatom strains exhibited high photo-physiological plasticity and eurythermal traits that would allow for survival in both the Baltic Sea and the peatlands. A storm surge driven intermixing process of water masses from both ecosystems would generally facilitate diatom growth, but only in the presence of sufficient salinity for Baltic Sea isolates. More importantly, our data clearly show that peatland water enhances growth with the indication of heterotrophic growth. Overall, benthic diatoms from the Baltic Sea and the adjacent peatlands are very well acclimated to respond to the described intermixing processes along the terrestrial-marine ecocline resulting from sea level rise and storm surges.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microorganisms10040749/s1>, Figure S1: Identification plate with scanning electron microscopy images (by Kana Kuriyama) of the frustules of *Hyalodiscus scoticus*. Figure S2: Light microscopy images of the vital cell *Hyalodiscus scoticus*. Figure S3: Scanning electron

microscopy images of the frustules of *Hyalodiscus scoticus*. Figure S4: Scanning electron microscopy images of the frustules of (A), (B) *Melosira nummuloides*, (C), (D) *Nitzschia filiformis*, (E) *Planothidium* sp. (st. 1), (F) *Planothidium* sp. (st. 2). Table S1: Confidence intervals for temperature-dependent photosynthetic and respirational rates of two benthic Baltic Sea diatom strains. Table S2: Confidence intervals for temperature-dependent photosynthetic and respirational rates of three benthic peatland diatom strains.

Author Contributions: L.R.P. and U.K. developed the idea and elaborated on the concept; L.R.P. provided experimental and taxonomical data; L.R.P. provided all statistical data, conducted the data analyses, and provided the first draft, which was critiqued and edited by U.K. All authors have read and agreed to the published version of the manuscript.

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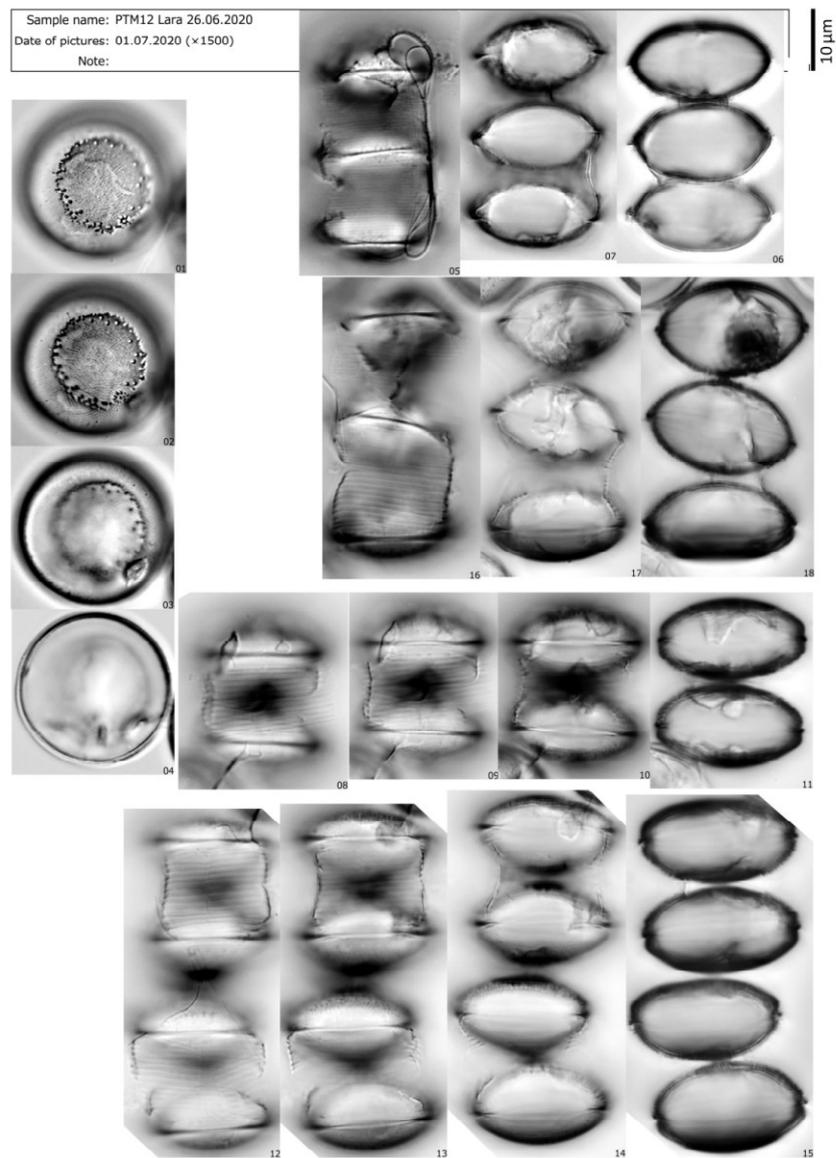


Figure S1. Identification plate with scanning electron microscopy images (by Kana Kuriyama) of the frustules of *Hyalodiscus sordicus*

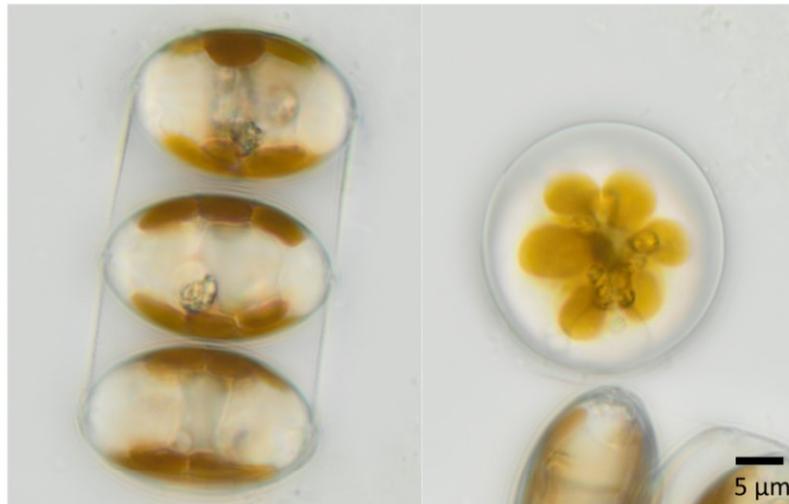


Figure S2. Light microscopy images of the vital cell *Hyalodiscus scoticus*.

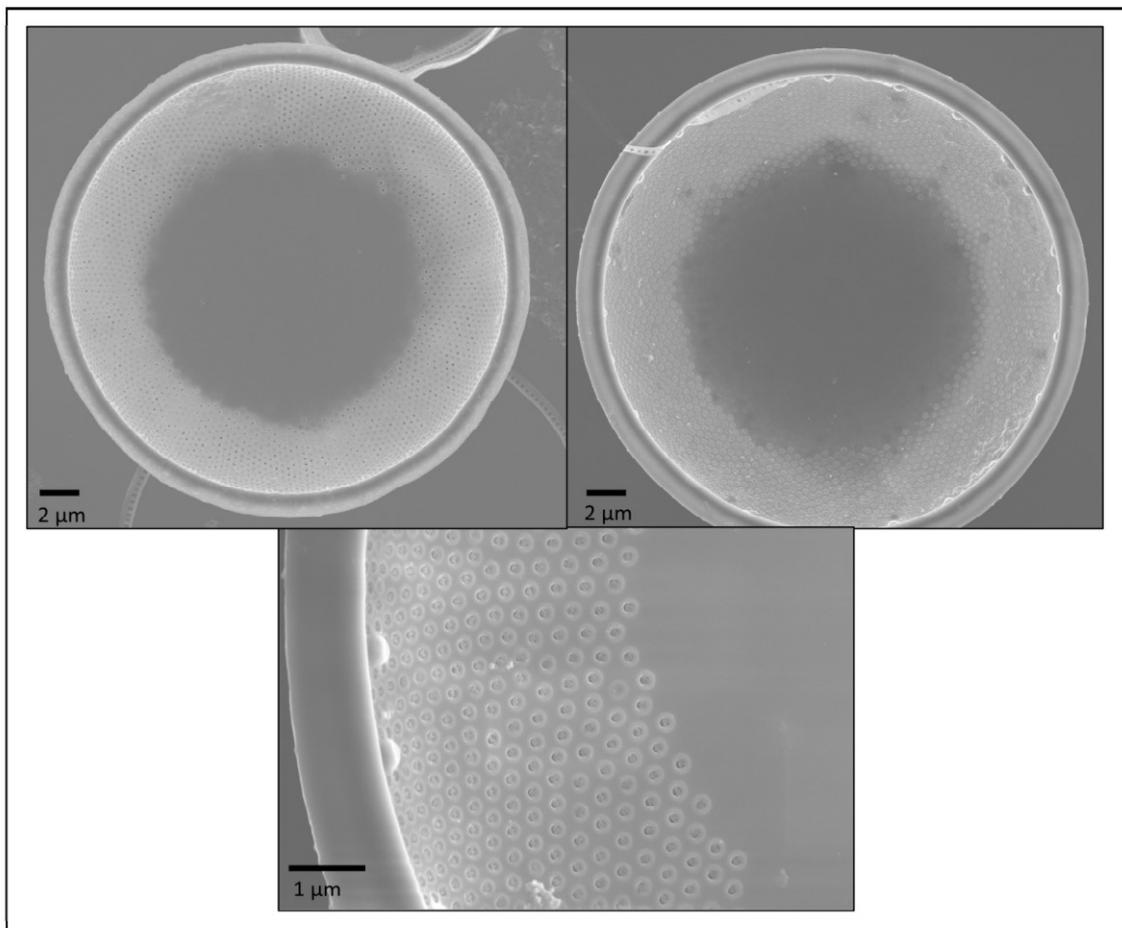


Figure S3. Scanning electron microscopy images of the frustules of *Hyalodiscus scoticus*.

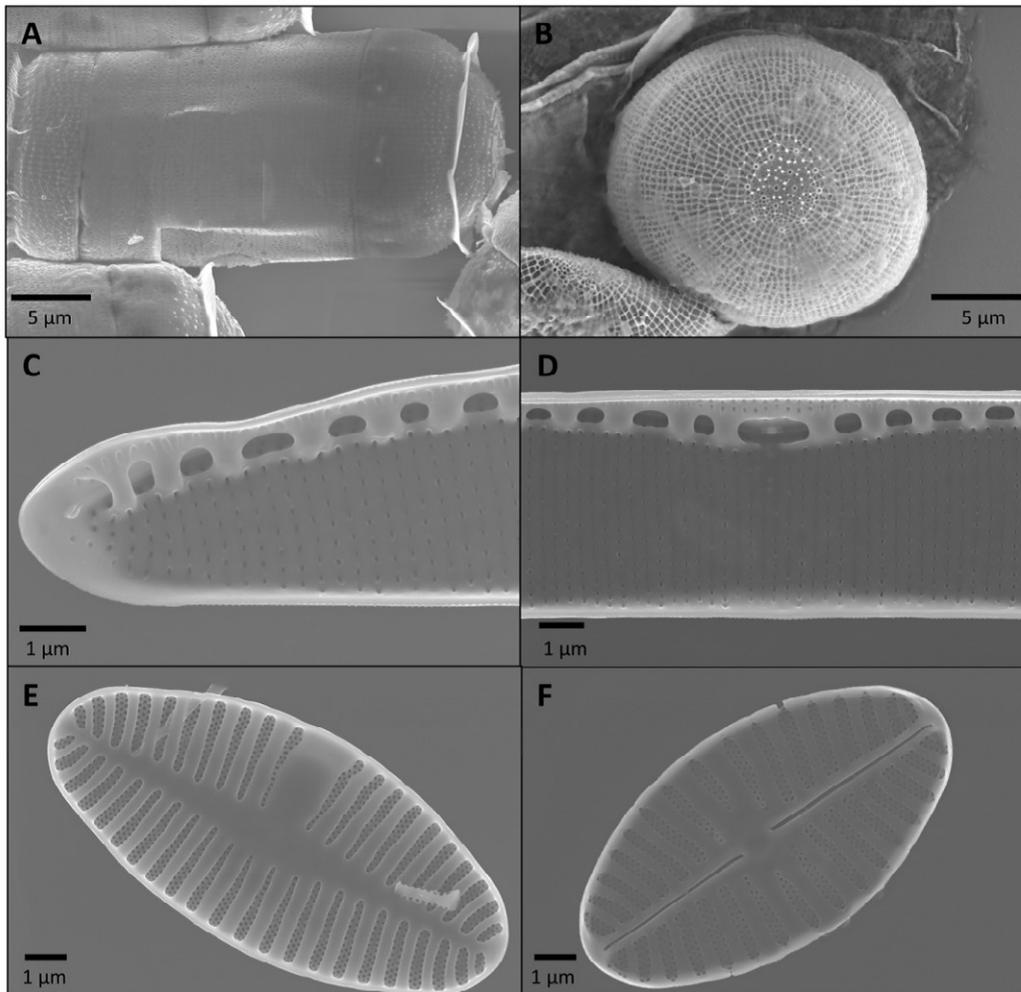


Figure S4. Scanning electron microscopy images of the frustules of (A), (B) *Melosira nummuloides*, (C), (D) *Nitzschia filiformis*, (E) *Planothidium* sp. (st. 1), (F) *Planothidium* sp. (st. 2).

Table S1 Confidence intervals for temperature-dependent photosynthetic and respiratory rates of two benthic Baltic Sea diatom strains.

	2.5%	97.5%
<i>Melosira nummuloides</i> GM6		
maximal photosynthetic rate	120.985	168.020
optimum photosynthetic temperature	4.327	13.716
maximum photosynthetic temperature	32.089	36.719
maximum respiratory rate	-115.842	-85.969
optimum respiratory temperature	33.163	36.112
maximum respiratory temperature	41.404	47.804
<i>Melosira nummuloides</i> GM9		
maximal photosynthetic rate	93.627	158.208
optimum photosynthetic temperature	-0.734	13.395
maximum photosynthetic temperature	30.529	36.984
maximum respiratory rate	-77.547	-51.426
optimum respiratory temperature	32.150	41.078
maximum respiratory temperature	37.659	57.744
<i>Hyalodiscus cf. scoticus</i> GM6		
maximal photosynthetic rate	48.900	99.277
optimum photosynthetic temperature	-0.340	8.654
maximum photosynthetic temperature	20.324	24.650
maximum respiratory rate	-108.003	-73.185
optimum respiratory temperature	31.596	35.049
maximum respiratory temperature	40.474	46.239
<i>Hyalodiscus cf. scoticus</i> GM9		
maximal photosynthetic rate	96.801	200.864
optimum photosynthetic temperature	11.297	25.130
maximum photosynthetic temperature	32.675	40.195
maximum respiratory rate	-50.414	-34.560
optimum respiratory temperature	31.651	35.768
maximum respiratory temperature	40.474	49.443

Table S2 Confidence intervals for temperature-dependent photosynthetic and respirational rates of three benthic peatland diatom strains.

	2.5%	97.5%
<i>Planothidium</i> sp. (st. 2) GM6		
maximal photosynthetic rate	50.027	64.185
optimum photosynthetic temperature	18.060	23.161
maximum photosynthetic temperature	38.377	42.978
maximum respirational rate	-51.693	-39.228
optimum respirational temperature	31.314	34.649
maximum respirational temperature	41.690	49.978
<i>Planothidium</i> sp. (st. 2) GM9		
maximal photosynthetic rate	64.016	88.145
optimum photosynthetic temperature	16.519	23.643
maximum photosynthetic temperature	38.332	46.517
maximum respirational rate	-41.846	-29.968
optimum respirational temperature	31.433	36.698
maximum respirational temperature	40.586	53.530
<i>Nitzschia filiformis</i> GM6		
maximal photosynthetic rate	63.125	112.760
optimum photosynthetic temperature	16.010	28.221
maximum photosynthetic temperature	34.995	55.693
maximum respirational rate	-86.450	-64.999
optimum respirational temperature	32.818	37.946
maximum respirational temperature	41.237	53.458
<i>Nitzschia filiformis</i> GM9		
maximal photosynthetic rate	97.391	179.846
optimum photosynthetic temperature	10.977	25.748
maximum photosynthetic temperature	34.082	50.310
maximum respirational rate	-54.806	-44.706
optimum respirational temperature	32.763	34.856
maximum respirational temperature	42.381	46.782
<i>Planothidium</i> sp. (st. 1) GM6		
maximal photosynthetic rate	85.206	101.016
optimum photosynthetic temperature	24.474	26.957
maximum photosynthetic temperature	38.861	40.533
maximum respirational rate	-51.439	-42.739
optimum respirational temperature	33.157	37.708
maximum respirational temperature	43.553	54.557
<i>Planothidium</i> sp. (st. 1) GM9		
maximal photosynthetic rate	103.504	123.181
optimum photosynthetic temperature	24.846	27.405
maximum photosynthetic temperature	39.625	41.730
maximum respirational rate	-48.406	-40.454
optimum respirational temperature	33.080	35.243
maximum respirational temperature	43.048	48.022

5.1 Declaration of own contribution to the publications and manuscripts

Publication I

Lara R. Prella, Angelika Graiff, Sigrid Gründling-Pfaff, and Ulf Karsten developed the idea and elaborated the concept. **Lara R. Prella**, Angelika Graiff, Sigrid Gründling-Pfaff, Veronika Sommer, and Kana Kuriyama provided experimental or taxonomic data. All authors organized and conducted the data analyses. **Lara R. Prella**, Angelika Graiff, and Sigrid Gründling-Pfaff wrote the first draft of the manuscript, which was commented and edited by all other authors.

Publication II

Lara R. Prella, Martin Albrecht, Ulf Karsten, and Karin Glaser developed the idea and elaborated the concept. **Lara R. Prella**, Martin Albrecht, Pauline Damer, Tabea Giese, Jessica Jähns, Simon Müller, Louisa Schulz, Lennard Viertel, and Karin Glaser provided experimental and taxonomic data. **All authors** organized and conducted the data analyses and were involved in writing the first draft of the manuscript, which was commented and edited by **Lara R. Prella**, Martin Albrecht, Ulf Karsten, and Karin Glaser and finally accepted by **all authors**.

Publication III

Lara R. Prella and Ulf Karsten developed the idea and elaborated the concept. **Lara R. Prella** provided experimental and taxonomical data. **Lara R. Prella** provided all statistical data, conducted the data analyses and provided the first draft, which was critiqued and edited by Ulf Karsten. **Both authors** approved the final version.

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5.3 Selbstständigkeitserklärung

Ich, Lara R. Prella, versichere hiermit an Eides statt, dass ich die vorliegende Arbeit selbstständig angefertigt und ohne fremde Hilfe verfasst habe. Dazu habe ich keine außer den von mir angegebenen Hilfsmitteln und Quellen verwendet und die den benutzten Werken inhaltlich und wörtlich entnommenen Stellen habe ich als solche kenntlich gemacht.

Rostock, den

Lara R. Prella

5.4 Curriculum Vitae

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Education

- 2019 - ... **PhD Student, Applied Ecology and Phycology, University of Rostock**
DFG Research Training Group Baltic TRANSCOAST, Subproject B2: Microphytobenthos
- 2015 - 2018 **Master of Science Marine Biology, University of Rostock**
- 2012 - 2015 **Bachelor of Science Biology, Carl von Ossietzky University Oldenburg**
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Publications (peer review)

Moorthi, S. D., Schmitt, J. A., Ryabov, A., Tsakalakis, I., Blasius, B., **Prella, L.**, Tiedemann, M. & Hodapp, D. (2016). Unifying ecological stoichiometry and metabolic theory to predict production and trophic transfer in a marine planktonic food web. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371 (1694), 20150270.

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Scientific oral and poster presentations in (international) symposia

Prelle, L., Graiff, A., Gründling-Pfaff, S., Sommer, V., Kuriyama, K. and Karsten, U. (2020). Ecophysiological response of Baltic Sea benthic diatoms to changing environmental conditions with emphasis to sea-land exchange processes. 18th Conference of the Phycology Section in the German Society for Plant Sciences (DBG): Kall-Steinfeld. 8-11 März 2020. Posterpresentation and 2nd winner of poster price.

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